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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appellant(s):	Walke *et al.*	Group Art Unit:	1646
Application No.:	09/819,946	Examiner:	M. T. Brannock
Filed:	03/28/01		
Title:	Novel Human 7TM Proteins and Polynucleotides Encoding the Same	Atty. Docket No.	LEX-0157-USA

APPEAL BRIEF

Mail Stop Appeal Brief
Commissioner for Patents
Alexandria, VA 22313

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APPEAL BRIEF

Sir:

Appellants hereby submit an original and two copies of this Appeal Brief to the Board of Patent Appeals and Interferences ("the Board") in response to the Final Office Action mailed January 10, 2003. The Notice of Appeal was timely submitted on April 10, 2003, and was received in the Patent and Trademark Office ("the Office") on April 15, 2003. This Appeal Brief is timely submitted in light of the concurrently filed Petition for an Extension of Time of five months to and including November 15, 2003 which falls on a Saturday and is therefore extended to Monday, November 17, 2003 and authorization to deduct the fee as required under 37 C.F.R. § 1.17(a)(2) from Appellants' Representatives' deposit account. The Commissioner is also authorized to charge the fee for filing this Appeal Brief (**$165.00**), as required under 37 C.F.R. § 1.17(c), to Lexicon Genetics Incorporated Deposit Account No. 50-0892.

Appellants believe no fees in addition to the fee for filing the Appeal Brief and the fee for the extension of time are due in connection with this Appeal Brief. However, should any additional fees under 37 C.F.R. §§ 1.16 to 1.21 be required for any reason related to this communication, the Commissioner is authorized to charge any underpayment or credit any overpayment to Lexicon Genetics Incorporated Deposit Account No. 50-0892.

I. REAL PARTY IN INTEREST

The real party in interest is the Assignee, Lexicon Genetics Incorporated, 8800 Technology Forest Place, The Woodlands, Texas, 77381.

II. RELATED APPEALS AND INTERFERENCES

Appellants know of no related appeals or interferences.

III. STATUS OF THE CLAIMS

The present application was filed on March 28, 2001, claiming the benefit of U.S. Provisional

Application Number 60/192,978, which was filed on March 28, 2000, and included original claims 1-5. A Restriction and Election Requirement was issued by the Office on June 11, 2002, via telephone conference with Peter Seferian, restricting to a particular invention. In response to the Restriction Requirement, Appellants elected, without traverse, the claims of the Group I invention (comprising original claims 1-3) for prosecution on the merits and claims 4-5 are withdrawn from further consideration by the Examiner, as being drawn to a non-elected invention. A First Official Action, was issued on June 19, 2002 ("the First Action"), claim 2 was rejected under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite, claims 1-3 were rejected under 35 U.S.C. § 101, due to the alleged lack of patentable utility, claims 1-3 were also rejected under 35 U.S.C. § 112, first paragraph, as allegedly unusable by the skilled artisan due to the alleged lack of patentable utility. In a response to the First Official Action, submitted to the Office on October 17, 2002 ("response to the First Action"), Appellants amended claims 1 and 2 and added new claims 6 and 7 to better claim the present invention and traversed the rejection of the pending claims under 35 U.S.C. § 101 and 35 U.S.C. § 112, first paragraph. A Second and Final Official Action, was issued on January 10, 2003 (the "Final Action"), in which rejection of Claim 2 was maintained under 35 U.S.C. §112, second paragraph, as allegedly being indefinite. The rejection of claims 1-3, 6 and 7 was maintained under 35 U.S.C. § 101 as allegedly lacking a patentable utility and under 35 U.S.C. § 112, first paragraph, as allegedly one skilled in the art clearly would not know how to use the skilled invention. In a response to the Final Action, submitted on April 10, 2003 ("response to the Final Action"), claim 2 was further amended for clarification and Appellants again traversed the outstanding rejections of claims 1-3, 6 and 7under under 35 U.S.C. § 101 and § 112, first paragraph. An Advisory Action ("the Advisory Action") was mailed on June 3, 2003, withdrawing the rejection of claim 2 under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite, but maintaining the rejection of claims 1-3, 6 and 7 under 35 U.S.C. § 101, as allegedly lacking a patentable utility and under 35 U.S.C. § 112, first paragraph, as one skilled in the art clearly would not know how to use the skilled invention. Appellants filed a Notice of Appeal on April 10, 2003 which was received at the PTO on April 15, 2003. A copy of the appealed claims is included below in the Appendix (Section IX).

IV. STATUS OF THE AMENDMENTS

For the purposes of Appeal Appellants believe that no additional outstanding amendments exist.

V. SUMMARY OF THE INVENTION

The present invention relates to Appellants' discovery and identification of novel human sequences that encode a human G protein-coupled receptor known as *Homo sapiens* taste receptor (TASR1), a protein of known function. Also disclosed are naturally occurring polymorphisms that exist within these molecules (page 7, line 27-page 8, line5). The specification details a number of uses for the presently claimed sequences, including the detection and diagnosis of human diseases such as, *inter alia*, obesity (page 57, line 31 through page 58, line 1) wherein such molecules can act as drug targets (specification at page 2, line 3 and page 11, lines 12-16). Appellants have used the methods described in the specification as filed (page 2, lines 21-27, page 28 ,line 4 and page 29, line 8) to construct knockout mice which were used to biologically validate the assertions that the sequences of the present invention have utility as drug targets for human diseases, such as obesity (specification at page 58, line 1). Additional uses for the sequences of the present invention include assessing temporal and tissue specific gene expression patterns (page 10, lines 8-10), particularly using a high throughput "chip" format (specification at page 9, line 16 through page 11), mapping the sequences to a specific region of a human chromosome (specification at page 4 lines 2-4) and identifying protein encoding regions, determining the genomic structure (specification at page 14, line 18-26), and in diagnostic assays such as forensic analysis, human population biology and paternity determinations (see, for example, the specification page 14, line 21, page 18, line 25, page 30, line 33, page 33, line 31, page 34, line 14) wherein the sequences of the present invention are particularly useful as the specification identified polymorphisms (page 7, line 27 - page 8, line5) that can be used in these assays. Thus, Appellants have described novel nucleic and amino acid sequences and naturally occurring polymorphisms that exist within the sequences of the present invention. The sequences of the present invention encode a human G protein-coupled receptor known as *Homo sapiens* taste receptor (TASR1), a protein of known function and Appellants have used methods described in the specification as filed to biologically validate their credible assertions that the sequence of the present invention have utility

as drug targets for human diseases such as obesity as well as other utilities.

VI. ISSUES ON APPEAL

1. Do claims 1-3, 6 and 7 lack a patentable utility?
2. Are claims 1-3, 6 and 7 unusable by a skilled artisan due to a lack of patentable utility?

VII. GROUPING OF THE CLAIMS

For the purposes of the outstanding rejections under 35 U.S.C. § 101 and 35 U.S.C. § 112, first paragraph, the claims will stand or fall together.

VIII. ARGUMENT

A. Do Claims 1-3, 6 and 7 Lack a Patentable Utility?

The Final Action rejected and the Advisory Action maintained the rejection of claims 1-3, 6 and 7 under 35 U.S.C. § 101, as allegedly lacking a patentable utility due to not being supported by either a specific and substantial utility or a well-established utility, this rejection is maintained in the Advisory Action.

Appellants have described novel human sequences that encode a human G protein-coupled receptor known as *Homo sapiens* taste receptor (TASR1), a protein of known function. Also disclosed are naturally occurring polymorphisms that exist within these molecules (page 7, line 27-page 8, line5). The specification details a number of uses for the presently claimed sequences, including the detection and diagnosis of human diseases such as, *inter alia*, obesity (page 57, line 31 through page 58, line 1) wherein such molecules can act as drug targets (specification at page 2, line 3 and page 11, lines 12-16). Recently, Appellants have used the methods described in the specification as filed (page 2, lines 21-27, page 28, line 4 and page 29, line 8) to construct knockout mice which were used to biologically validate the assertions that the sequences of the present invention have utility as drug targets for human diseases, such as obesity (specification at page 58, line 1). Appellants therefore asserted that the sequences of the present invention encode a human G protein-coupled receptor, a taste receptor (specification at page 5, line 4). A human

G protein-coupled taste receptor identified by those of skill in the art, in no way affiliated with Appellants, as *Homo sapiens* taste receptor (TASR1). Therefore, clearly Appellant's assertions were credible and further recent findings (described below) made by Appellants using the methods described in the specification as filed of making knockout animals, provide proof that their identity and disease assertions were correct by biologically validating Appellants assertions that the sequences of the present invention have utility as drug targets for human disease. Thus clearly the sequences of the present invention have patentable utility and pending rejections under 35 U.S.C. § 101 and 35 U.S.C. § 112, first paragraph should be overturned.

First, as set forth in the response to the First Action and the response to the Final Action, Appellants would like to invite the Board's attention to the fact that a sequence that is nearly identical at the amino acid level over the entire length of SEQ ID NO: 2 of the present invention is present in the leading scientific repository for biological sequence data (GenBank), and has been annotated by third party scientists *wholly unaffiliated with Appellants* as *Homo sapiens* taste receptor (TASR1) (GenBank accession number NP_619642, alignment and GenBank report provided in **Exhibit A**). Therefore, it is clear that the amino acid sequence of SEQ ID NO:2 encodes a human G protein-coupled receptor, taste receptor TASR1. This clearly supports Appellant's assertion that those of skill in the art would recognize the present invention as a human G protein-coupled receptor and a taste receptor. Therefore, the scientific evidence of identity at the amino acid level clearly establishes that those of skill in the art would recognize the sequences of the present invention as a human G protein-coupled receptor and a taste receptor, TASR1, a protein with known function. Therefore, Appellants have described a utility in full compliance with the provisions of 35 U.S.C. section 101, and the Examiner's rejection should be overturned.

Given this clear evidence that those of skill in the art would recognize the present invention as a G protein-coupled receptor, more specifically taste receptor (TASR1), which is described in the publication submitted previously in the response to the First Action, entitled "Human receptors for sweet and umami taste" (Li, et al., PNAS 99(7):4692-6, 2002, **Exhibit B**). Clearly, there can be no question that Appellants' asserted utility for the described sequences is "credible." Appellants have thus supplied evidence supporting their assertion that those of skill in the art would recognize that the sequences of the

present invention encode a G protein-coupled receptor, in particular that of taste receptor (TASR1).. In contrast, the Examiner has provided no evidence of record indicating that those of skill in the art would not recognize the sequences of the present invention encode a G protein-coupled receptor. As such, the scientific evidence clearly establishes that Appellants have described an invention whose utility is in full compliance with the provisions of 35 U.S.C. § 101, and the Examiner's rejection should be withdrawn.

Furthermore, it is well known to the art that novel human G-protein coupled receptors have a well-established utility. This is evidenced by the fact that fully 60% of licensed drugs target G-protein coupled receptors (Gurrath, 2001, Curr. Med. Chem. *8*:1605-1648: **Exhibit C**). In addition, Appellants' assertion, that the presently described sequences have specific, credible and well-established utility, is also supported by the fact that multiple millions of dollars are allocated yearly in the identification and targeting of G protein-coupled receptors such as those of present invention. If these molecules did not have well-established utility recognized by those of skill in the art in the pharmaceutical industry, surely those in such a competitive industry would not direct so much of their limited resources towards this class of receptors.

Additionally, methods similar to those of the present invention were used to identify the G protein-coupled receptors of issued U.S. Patent 6,043,052 (**Exhibit D**). Issued U.S. Patents are presumed to be valid and to meet the requirements of 35 U.S.C. §§ 101, 102, 103 and 112, specifically, that they have utility, are novel, non-obvious, are enabled, meet the written description requirements and particularly point out and distinctly claim the invention. Therefore, the Appellants' assertion that the described G protein-coupled receptors is in fact a G protein-coupled receptors is supported by issued U.S. Patent 6,043,052, as well as the plethora of other G protein- coupled receptor patents that the office has issued. For example, the specific and substantial utility of human G protein- coupled receptors is evidenced by the fact that they are the subject of the above mentioned U.S. Patent No. 6,043,052 which discloses polynucleotides encoding a novel G protein- coupled receptor and U.S. Patent Nos. 5,891,646 (**Exhibit E**) and 6,110,693 (**Exhibit F**), both of which disclose and claim methods for detecting G protein- coupled receptor activity *in vivo* and *in vitro*, methods for assaying G protein- coupled receptor activity, and methods of screening for G protein- coupled receptor ligands, G protein- coupled receptor kinase activity, components that interact with G protein- coupled receptor regulatory processes and constructs useful in

such methods. The issuance of these U.S. patents clearly indicates that G protein- coupled receptor polynucleotides have utility and that such utilities were sufficiently specific and substantial to warrant the issuance of U.S. patents directed to methods used to identify and characterize G protein- coupled receptors. The teachings of these patentable disclosures are directly applicable to the present invention (G protein- coupled receptor polynucleotides) and are evidence that those skilled in the art recognize the specific and substantial utility of G protein- coupled receptors. In light of the issuance of U.S. Patent No. 6,043,052 on polynucleotides encoding a novel G protein- coupled receptor, Appellants respectfully submit that the present application, which also describes polynucleotides encoding a novel G protein- coupled receptor, describes an invention with specific and substantial utility fully compliant with 35 U.S.C. § 101.

Additionally, clearly the evidence supports Appellants' assertions that the sequences of the present invention encode a novel human G protein-coupled receptor, a taste receptor - TASR1, which has a utility that is recognized by those of skill in the art. Thus, this situation is similar to Example 10 of the PTO's Revised Interim Utility Guidelines Training Materials (pages 53-55), which establishes that a rejection under 35 U.S.C. § 101 as allegedly lacking a patentable utility and under 35 U.S.C. § 112, first paragraph as allegedly unusable by the skilled artisan due to the alleged lack of patentable utility, is not proper when there is no reason to doubt the asserted utility of a full length sequence (such as the presently claimed sequence) that has a similarity to a protein having a known function. In the Analysis portion of Example 10 it states that "Based on applicant's disclosure and the results of the PTO search, there is no reason to doubt the assertion that SEQ ID NO:2 encodes a DNA ligase . Further DNA ligases have a well-established use in the molecular biology art based on this class of proteins ability to ligate DNA. .....Note that if there is a well-established utility already associated with the claimed invention, the utility need not be asserted in the specification as filed..... Thus the conclusion reached from this analysis is that a 35 U.S.C. § 101 and a 35 U.S.C. § 112 first paragraph, utility rejection should not be made."

The present case is similar to that presented in Example 10 of the Revised Interim Utility Guidelines Training Materials (pages 53-55). In the present case it is clear that the sequences of the present invention encode a novel human G protein-coupled receptor (taste receptor, TASR1): TASR1 has well-established utility. According to the guidelines "Note that if there is a well-established utility already associated with

the claimed invention, the utility need not be asserted in the specification as filed...Thus the conclusion reached from this analysis is that a 35 U.S.C. § 101 and a 35 U.S.C. § 112 first paragraph, utility rejection should not be made." Thus the rejection of the presently claimed invention under a 35 U.S.C. § 101 and a 35 U.S.C. § 112 first paragraph utility rejection should be overruled.

In addition, Appellants have recently acquired evidence involving the analysis of transgenic "knockout" mice, in which the function of the mouse homolog of the gene encoding the sequences of the present invention was disrupted in embryonic stem cells (constructed as described in the specification at least at page 2, lines 21-27, page 28 ,line 4 and page 29, line 8). Knockout mice prepared as described in the specification as filed were subject to a medical work-up using an integrated suite of medical diagnostic procedures designed to assess the function of the major organ systems in a mammalian subject. Disruption of the mouse gene of the present invention and thus elimination of the protein it encodes, resulted in a average 15% decrease in the body weight of homozygous knockout mice as compared with their wild-type littermates at 16 weeks of age. Clearly as knockout of the activity of the protein encoded by the sequences of the present invention resulted in decreased body weight, this protein and the sequences that encode it are logical drug targets for antagonists directed at treating obesity, among other related human disorders. This clearly provides additional evidence that as asserted in the Application as filed, the sequences of the present invention encode a molecule having biological function and utility. The sequences of the present invention and the molecule they encode, as well as agonists or antagonists directed at the them can be used to diagnose and treat, *inter alia*, obesity (as stated in the specification at least at specification at page 58, line 1), and thus are biologically validated drug targets. Thus clearly this finding supports Appellant's assertions that the molecules of the present invention also have real world substantial and specific utility, which in addition to the previously submitted evidence prove that the sequences of the present invention have utility, having been identified as biologically validated drug targets using methods and identified for diseases and disorders asserted in the specification as filed.

Thus, the skilled artisan would readily appreciate the utilities asserted by Appellants' regarding the role of the proteins encoded by sequences of the present invention, including those associated with diseases that have been linked to the novel human G protein-coupled receptor, taste receptor TASR1. Therefore,

the present utility rejection must fail. According to the Examination Guidelines for the Utility Requirement, if the applicant has asserted that the claimed invention is useful for any particular purpose (i.e., it has a "specific and substantial utility") and the assertion would be considered credible by a person of ordinary skill in the art, the Examiner should not impose a rejection based on lack of utility (66 Federal Register 1098, January 5, 2001).

The Advisory Action maintains that Appellants' additional assertions regarding the use of the presently claimed polynucleotides on DNA gene chips, based on the position that such a use would allegedly be generic. The Examiner seems to be requiring Appellants to identify the biological role of the nucleic acid or function of the protein encoded by the presently claimed polynucleotides before the present sequences can be used in gene chip applications that meet the requirements of § 101. Appellants respectfully point out that knowledge of the exact function or role of the presently claimed sequence is not required to track expression patterns using a DNA chip. As set forth in Appellants First Response, given the widespread utility of such "gene chip" methods using *public domain* gene sequence information, there can be little doubt that the use of the presently described *novel* sequences would have great utility in such DNA chip applications.

However in fact, Appellants have identified the biological role of the protein encoded by the sequences of the present invention, as a human G protein-coupled taste receptor (TASR1). Clearly given the extensive utility described above for the molecules encoded by the sequences of the present invention and evidence that the claimed sequences provide a specific marker of the gene encoding a human G protein-coupled taste receptor (TASR1) and provide a unique identifier of the corresponding gene in the human genome. Such specific markers are targets for discovering drugs that are associated with human disorders and diseases such as, *inter alia*, obesity (specification at page 58, line 1). Thus, those skilled in the art would instantly recognize that the present nucleotide sequence would be an ideal, novel candidate for assessing gene expression using, for example, DNA chips, as the specification details at least on specification at page 9, line 16 through page 11. Such "DNA chips" clearly have utility, as evidenced by hundreds of issued U.S. Patents, exemplified by U.S. Patent Nos. 5,445,934 (**Exhibit G**), 5,556,752 (**Exhibit H**), 5,744,305 (**Exhibit I**), as well as more recently issued U.S. Patent Nos. 5,837,832 (**Exhibit**

J), 6,156,501 (**Exhibit K**) and 6,261,776 (**Exhibit L**).

The Board is further requested to consider that, given the huge expense of the drug discovery process, even negative information has great "real world" practical utility. Knowing that a given gene is not expressed in medically relevant tissue provides an informative finding of great value to industry by allowing for the more efficient deployment of expensive drug discovery resources. Such practical considerations are equally applicable to the scientific community in general, in that time and resources are not wasted chasing what are essentially scientific dead-ends (from the perspective of medical relevance). Clearly, compositions that enhance the utility of such DNA gene chips, such as the presently claimed sequences encoding a human G protein-coupled taste receptor (TASR1) which Appellants have shown is a biologically validated drug target for obesity, among others, must in themselves be useful. Moreover, the presently described human G protein-coupled taste receptor (TASR1) provides uniquely specific sequence resources for identifying and quantifying full length transcripts that were encoded by the corresponding human genomic locus. Accordingly, there can be no question that the described sequences provide an exquisitely specific utility for analyzing gene expression.

The utility of the sequences of the present invention is further enhanced by the description in the specification of polymorphisms (page 7, line 27-page 8, line 5). These teachings along with the above evidence that the molecules of the present invention encode a human G protein-coupled taste receptor (TASR1), a protein of known function and that Appellants have used methods described in the specification as filed to biologically validate their assertions that the sequence of the present invention have utility as drug targets for human disease, clearly demonstrate outstanding utility of the sequences in DNA chip expression analysis.

Still further, as only a small percentage of the genome (2-4%) actually encodes exons, which in turn encode amino acid sequences. Thus, not all human genomic DNA sequences are useful in such gene chip applications. This further discounts the Examiner's position that such uses are "generic". The present claims clearly meet the requirements of 35 U.S.C. § 101. It has been clearly established that a statement of utility in a specification must be accepted absent reasons why one skilled in the art would have reason to doubt the objective truth of such statement. *In re Langer*, 503 F.2d 1380, 1391, 183 USPQ 288, 297

(CCPA, 1974); *In re Marzocchi*, 439 F.2d 220, 224, 169 USPQ 367, 370 (CCPA, 1971).

Additional evidence of the "real world" substantial utility of the present invention is further provided by the fact that there is an entire industry based on the use of gene sequences or fragments thereof in a gene chip format. Perhaps the most notable gene chip company is Affymetrix. However, there are many companies which have, at one time or another, concentrated on the use of gene sequences or fragments, in gene chip and non-gene chip formats, for example: Gene Logic, ABI-Perkin-Elmer, HySeq and Incyte. In addition, one such company, Rosetta Inpharmatics, was viewed to have such "real world" value that it was acquired by large pharmaceutical company, Merck & Co., for substantial sums of money (net equity value of the transaction was $620 million). The "real world" substantial industrial utility of gene sequences or fragments would, therefore, appear to be widespread and well established. Clearly, persons of skill in the art, as well as venture capitalists and investors, readily recognize the utility, both scientific and commercial, of genomic data in general, and specifically human genomic data. Billions of dollars have been invested in the human genome project, resulting in useful genomic data (see, *e.g.*, Venter *et al.*, 2001, Science *291*:1304; **Exhibit M**). The results have been a stunning success as the utility of human genomic data has been widely recognized as a great gift to humanity (see, *e.g.*, Jasny and Kennedy, 2001, Science *291*:1153; **Exhibit N**). Clearly, the usefulness of human genomic data, such as the presently claimed nucleic acid molecules, is substantial and credible (worthy of billions of dollars and the creation of numerous companies focused on such information) and well-established (the utility of human genomic information has been clearly understood for many years).

As a still further example of utility is the use of the present sequences in such diagnostic assays (see, for example, the specification page 14, line 21, page 18, line 25, page 30, line 33, page 33, line 31, page 34, line 14) as those associated with identification of paternity and forensic analysis, among others. The sequences of the present invention have particular utility as the application as filed identified several polymorphisms (page 7, line 27-page 8, line 5). This is also not a case of a potential utility. Appellants respectfully submit that even in the worst case scenario, the described polymorphisms are each useful to distinguish 50% of the population (in other words, the marker being present in half of the population) and that the ability of a polymorphic marker to distinguish at least 50% of the population is an inherent feature

of any polymorphic marker, and this feature is well understood by those of skill in the art. Appellants note that as a matter of law, it is well settled that a patent need not disclose what is well known in the art. *In re Wands*, 8 USPQ 2d 1400 (Fed. Cir. 1988). Appellants support for Appellants' assertion of utility is provided by the fact that the skilled artisan would readily recognize and easily believe that the presently described polymorphic markers could be useful in forensic analysis. The fact that forensic biologists use polymorphic markers such as those described by Appellants every day provides more that ample support for the assertion that forensic biologists would also be able to use the specific polymorphic markers described by Appellants in the same fashion. Therefore, again it is clear that the sequences of the present invention have utility.

Given the physiologic activity and importance of human G protein-coupled taste receptor (TASR1) as known to those of skill in the art, those of skill in the art would readily appreciate the importance of tracking the expression of the genes encoding the described proteins, particularly due to the established role of TASR1 in human disorders such as such as obesity (specification at page 58, line 1). The use of the claimed polypeptide in an array for screening purposes Appellants respectfully point out that nucleic acid sequences have the greatest specific utility in gene chip applications once the role of the sequence has been identified, as have tissues of interest, as in the present case. Once the role of the particular nucleic acid is known, the level of gene expression has and even greater significance. By identifying the physiological activity role of the claimed sequence, the claimed sequence has a far greater utility in gene chip applications that just any random piece of DNA. Appellants respectfully submit that **specific** utility, which is the proper standard for utility under 35 U.S.C. § 101, is distinct from the requirement for a **unique** utility, which is clearly an improper standard. As clearly stated by the Federal Circuit in *Carl Zeiss Stiftung v. Renishaw PLC*, 20 USPQ2d 1101 (Fed. Cir. 1991; "*Carl Zeiss*"):

> An invention need not be the best or only way to accomplish a certain result, and it need only be useful to some extent and in certain applications: "[T]he fact that an invention has only limited utility and is only operable in certain applications is not grounds for finding a lack of utility." *Envirotech Corp. v. Al George, Inc.*, 221 USPQ 473, 480 (Fed. Cir. 1984)

Therefore, just because other nucleic acid sequences find utility in gene chip applications does not mean

that the use of Appellants' sequence in gene chip applications is not a specific utility. Furthermore, the requirement for a unique utility is clearly not the standard adopted by the Patent and Trademark Office. If every invention were required to have a unique utility, the Patent and Trademark Office would no longer be issuing patents on batteries, automobile tires, golf balls, golf clubs, and treatments for a variety of human diseases, such as cancer and bacterial or viral infections, just to name a few particular examples, because examples of each of these have already been described and patented. All batteries have the exact same utility - specifically, to provide power. All automobile tires have the exact same utility - specifically, for use on automobiles. All golf balls and golf clubs have the exact same utility - specifically, use in the game of golf. All cancer treatments have the exact same utility - specifically, to treat cancer. All anti-infectious agents have the exact same broader utility - specifically, to treat infections. However, only the briefest perusal of virtually any issue of the Official Gazette provides numerous examples of patents being granted on each of the above compositions every week. Furthermore, if a composition needed to be unique to be patented, the entire class and subclass system would be an effort in futility, as the class and subclass system serves solely to group such common inventions, which would not be required if each invention needed to have a unique utility. Thus, the present sequence clearly meets the requirements of 35 U.S.C. § 101.

Further evidence of utility of the presently claimed polynucleotide, although only one is needed to meet the requirements of 35 U.S.C. § 101 (*Raytheon v. Roper*, 220 USPQ 592 (Fed. Cir. 1983); *In re Gottlieb*, 140 USPQ 665 (CCPA 1964); *In re Malachowski*, 189 USPQ 432 (CCPA 1976); *Hoffman v. Klaus*, 9 USPQ2d 1657 (Bd. Pat. App. & Inter. 1988)), is the specific utility the present nucleotide sequence has in determining the genomic structure of the corresponding human chromosome (specification at page 4, lines 2-4) , for example mapping the protein encoding regions as described in the specification and evidenced below. Clearly, the present polynucleotide provides exquisite specificity in localizing the specific region of the human chromosome containing the gene encoding the given polynucleotide, a utility not shared by virtually any other nucleic acid sequence. In fact, it is this specificity that makes this particular sequence so useful. Early gene mapping techniques relied on methods such as Giemsa staining to identify regions of chromosomes. However, such techniques produced genetic maps with a resolution of only 5 to 10 megabases, far too low to be of much help in identifying specific genes involved in disease. The

skilled artisan readily appreciates the significant benefit afforded by markers that map a specific locus of the human genome, such as the present nucleic acid sequence.

Only a minor percentage of the genome actually encodes exons, which in turn encode amino acid sequences. The presently claimed polynucleotide sequence provides biologically validated empirical data (*e.g.*, showing which sequences are transcribed, spliced, and polyadenylated) that *specifically* defines that portion of the corresponding genomic locus that actually encodes exon sequence. Equally significant is that the claimed polynucleotide sequence defines how the encoded exons are actually spliced together to produce an active transcript (*i.e.*, the described sequences are useful for functionally defining exon splice-junctions). The Appellants respectfully submit that the practical scientific value of expressed, spliced, and polyadenylated mRNA sequences is readily apparent to those skilled in the relevant biological and biochemical arts. For further evidence supporting the Appellants' position, the Board is requested to review, for example, section 3 of Venter *et al.* (*supra at* pp. 1317-1321, including Fig. 11 at pp.1324-1325), which demonstrates the significance of expressed sequence information in the structural analysis of genomic data. The presently claimed polynucleotide sequence defines a biologically validated sequence that provides a unique and specific resource for mapping the genome essentially as described in the Venter *et al.* article.

As still further evidence of the specific utility of the sequences of the present invention in localizing the specific region of the human chromosome and identification of functionally active intron/exon splice junctions is the information provided in **Exhibit O**. This is the result of a blast analysis using SEQ ID NO:1 of the present invention when compared to the identified human genomic sequence. This result indicates that the sequence of the present invention is encoded by 6 exons spread non-contiguously along a region of human chromosome 1, which is contained within a region of the clone accession number AL591866.13 (Human DNA sequence from clone RP11-58A11 on chromosome 1). Thus clearly one would not simply be able to identify the protein encoding exons that make up the sequence of the present intention, nor to map the protein encoding regions identified specifically by the sequences of the present invention without knowing exactly what those specific sequences were.

In addition to the previously submitted Exhibits, demonstrating that the sequences of the present

invention encode a human G protein-coupled receptor, the taste receptor (TASR1). Genetic mapping of the sequences of the present invention maps to human chromosome 5, at the very same region as that to which TAS1R1: taste receptor, type 1, member 1 has been mapped. Therefore, in addition to the clear sequence homology between molecules annotated as human G protein-coupled taste receptor and the sequences of the present invention as has been demonstrated. The sequences of the present invention and the human G protein-coupled taste receptor, map to the same genetic locus, 1p36.23.

The question of utility is a straightforward one. As set forth by the Federal Circuit, "(t)he threshold of utility is not high: An invention is 'useful' under section 101 if it is capable of providing some identifiable benefit." *Juicy Whip Inc. v. Orange Bang Inc.*, 51 USPQ2d 1700 (Fed. Cir. 1999) (citing *Brenner v. Manson*, 383 U.S. 519, 534 (1966)). Additionally, the Federal Circuit has stated that "(t)o violate § 101 the claimed device must be totally incapable of achieving a useful result." *Brooktree Corp. v. Advanced Micro Devices, Inc.*, 977 F.2d 1555, 1571 (Fed. Cir. 1992), emphasis added. *Cross v. Iizuka* (224 USPQ 739 (Fed. Cir. 1985); "*Cross*") states "any utility of the claimed compounds is sufficient to satisfy 35 U.S.C. § 101". *Cross* at 748, emphasis added. Indeed, the Federal Circuit recently emphatically confirmed that "anything under the sun that is made by man" is patentable *(State Street Bank & Trust Co. v. Signature Financial Group Inc.*, 47 USPQ2d 1596, 1600 (Fed. Cir. 1998), citing the U.S. Supreme Court's decision in *Diamond vs. Chakrabarty*, 206 USPQ 193 (S.Ct. 1980)).

The legal test for utility simply involves an assessment of whether those skilled in the art would find any of the utilities described for the invention to be credible or believable. According to the Examination Guidelines for the Utility Requirement, if the applicant has asserted that the claimed invention is useful for any particular purpose (i.e., it has a "specific and substantial utility") and the assertion would be considered credible by a person of ordinary skill in the art, the Examiner should not impose a rejection based on lack of utility (66 Federal Register 1098, January 5, 2001).

In *In re Brana,* (34 USPQ2d 1436 (Fed. Cir. 1995), "*Brana*"), the Federal Circuit admonished the P.T.O. for confusing "the requirements under the law for obtaining a patent with the requirements for obtaining government approval to market a particular drug for human consumption". *Brana* at 1442. The Federal Circuit went on to state:

> At issue in this case is an important question of the legal constraints on patent office examination practice and policy. The question is, with regard to pharmaceutical inventions, what must the applicant provide regarding the practical utility or usefulness of the invention for which patent protection is sought. This is not a new issue; it is one which we would have thought had been settled by case law years ago.

Brana at 1439, emphasis added. The choice of the phrase "utility or usefulness" in the foregoing quotation is highly pertinent. The Federal Circuit is evidently using "utility" to refer to rejections under 35 U.S.C. § 101, and is using "usefulness" to refer to rejections under 35 U.S.C. § 112, first paragraph. This is made evident in the continuing text in *Brana*, which explains the correlation between 35 U.S.C. §§ 101 and 112, first paragraph. The Federal Circuit concluded:

> FDA approval, however, is not a prerequisite for finding a compound useful within the meaning of the patent laws. Usefulness in patent law, and in particular in the context of pharmaceutical inventions, necessarily includes the expectation of further research and development. The stage at which an invention in this field becomes useful is well before it is ready to be administered to humans. Were we to require Phase II testing in order to prove utility, the associated costs would prevent many companies from obtaining patent protection on promising new inventions, thereby eliminating an incentive to pursue, through research and development, potential cures in many crucial areas such as the treatment of cancer.

Brana at 1442-1443, citations omitted. In assessing the question of whether undue experimentation would be required in order to practice the claimed invention, the key term is "undue", not "experimentation". *In re Angstadt and Griffin*, 190 USPQ 214 (C.C.P.A. 1976). The need for some experimentation does not render the claimed invention unpatentable. Indeed, a considerable amount of experimentation may be permissible if such experimentation is routinely practiced in the art. *In re Angstadt and Griffin, supra*; *Amgen, Inc. v. Chugai Pharmaceutical Co., Ltd.*, 18 USPQ2d 1016 (Fed. Cir. 1991). As a matter of law, it is well settled that a patent need not disclose what is well known in the art. *In re Wands*, 8 USPQ 2d 1400 (Fed. Cir. 1988).

Finally, with regards to the issue of due process, while Appellants are well aware of the new Utility Guidelines set forth by the USPTO, Appellants respectfully point out that the current rules and regulations regarding the examination of patent applications is and always has been the patent laws as set forth in

35 U.S.C. and the patent rules as set forth in 37 C.F.R., not the Manual of Patent Examination Procedure or particular guidelines for patent examination set forth by the USPTO. Furthermore, it is the job of the judiciary, not the USPTO, to interpret these laws and rules. Appellants are unaware of any significant recent changes in either 35 U.S.C. § 101, or in the interpretation of 35 U.S.C. § 101 by the Supreme Court or the Federal Circuit that is in keeping with the new Utility Guidelines set forth by the USPTO. This is underscored by numerous patents that have been issued over the years that claim nucleic acid fragments that do not comply with the new Utility Guidelines. As examples of such issued U.S. Patents, the Board is invited to review U.S. Patent Nos. 5,817,479 (**Exhibit P**), 5,654,173 (**Exhibit Q**), and 5,552,281 (**Exhibit R**; each of which claims short polynucleotides), and recently issued U.S. Patent No. 6,340,583 (**Exhibit S**; which includes no working examples), none of which contain examples of the "real-world" utilities that the Examiner seems to be requiring. As issued U.S. Patents are presumed to meet all of the requirements for patentability, including 35 U.S.C. §§ 101 and 112, first paragraph (see Section VIII(B), below), Appellants submit that the present polynucleotides must also meet the requirements of 35 U.S.C. § 101. While Appellants agree that each application is examined on its own merits, Appellants are unaware of any changes to 35 U.S.C. § 101, or in the interpretation of 35 U.S.C. § 101 by the Supreme Court or the Federal Circuit, since the issuance of these patents that render the subject matter claimed in these patents, which is similar to the subject matter in question in the present application, as suddenly non-statutory or failing to meet the requirements of 35 U.S.C. § 101. Given the rapid pace of development in the biotechnology arts, it is difficult for the Appellants to understand how an invention fully disclosed and free of prior art at the time the present application was filed, could somehow retain *less* utility and be *less* enabled than inventions in the cited issued U.S. patents (which were filed during a time when the level of skill in the art was clearly lower). Simply put, Appellants invention is *more* enabled and retains *at least as much* utility as the inventions described in the claims of the U.S. patents of record. Thus, holding Appellants to a different standard of utility would be arbitrary and capricious, and, like other clear violations of due process, cannot stand.

Thus in summary, Appellants have described novel nucleic and amino acid sequences, their tissue expression pattern and naturally occurring polymorphisms that exist within these molecules. Furthermore,

the sequences of the present invention encode the human G protein-coupled taste receptor (TASR1), a protein of well recognized function. In addition, Appellants have used methods described in the specification as filed to biologically validate their assertions that the sequences of the present invention have utility as drug targets for human disease. Thus, the conclusion reached from this analysis is that a 35 U.S.C. § 101 and a 35 U.S.C. § 112 first paragraph, utility rejection should not have been made. Therefore, Appellants respectfully submit that the rejection of the presently claimed invention under a 35 U.S.C. § 101 and a 35 U.S.C. § 112 first paragraph utility rejection should be overruled.

B. Are Claims 1-3, 6 and 7 Unusable Due to a Lack of Patentable Utility?

The Final Action next rejects claims 1-3, 6 and 7 under 35 U.S.C. § 112, first paragraph, since allegedly one skilled in the art would not know how to use the invention, as the invention allegedly is not supported by either a clear asserted utility or a well-established utility.

The arguments detailed above in **Section VIII(A)** concerning the utility of the presently claimed sequences are incorporated herein by reference. As the Federal Circuit and its predecessor have determined that the utility requirement of Section 101 and the how to use requirement of Section 112, first paragraph, have the same basis, specifically the disclosure of a credible utility (*In re Brana, supra*; *In re Jolles*, 628 F.2d 1322, 1326 n.11, 206 USPQ 885, 889 n.11 (CCPA 1980); *In re Fouche*, 439 F.2d 1237, 1243, 169 USPQ 429, 434 (CCPA 1971)), Appellants submit that as claims 1-3, 6 and 7 have been shown to have "a specific, substantial, and credible utility", as detailed in **Section VIII(A)** above, the present rejection of claims 1-3, 6 and 7 under 35 U.S.C. § 112, first paragraph, cannot stand.

Appellants therefore submit that the rejection of claims 1-3, 6 and 7 under 35 U.S.C. § 112, first paragraph, must be overruled.

IX. APPENDIX

The claims involved in this appeal are as follows:

1. An isolated nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1.

2. An isolated nucleic acid molecule comprising a nucleotide sequence that:

(a) encodes the amino acid sequence of SEQ ID NO:2; and

(b) hybridizes under highly stringent conditions including washing in 0.1xSSC/0.1% SDS at 68°C to the complement of the nucleotide sequence of SEQ ID NO: 1.

3. An isolated nucleic acid molecule comprising a nucleotide sequence that encodes the amino acid sequence shown in SEQ ID NO:2.

6. An expression vector comprising a nucleic acid sequence encoding the amino acid sequence shown in SEQ ID NO: 2.

7. A cell comprising the expression vector of Claim 6.

X. CONCLUSION

Appellants respectfully submit that, in light of the foregoing arguments, the Final Action's conclusion that claims 1-3, 6 and 7 lack a patentable utility and are unusable by the skilled artisan due to a lack of patentable utility is unwarranted. It is therefore requested that the Board overturn the Final Action's rejections.

Respectfully submitted,

November 13, 2003
Date

Peter G. Sefenain Reg No 40162

Lance K. Ishimoto Reg. No. 41,866
Agent For Appellants

LEXICON GENETICS INCORPORATED
(281) 863-3399

Customer # 24231

```
 FASTA searches a protein or DNA sequence data bank
 version 3.3t05 March 30, 2000
Please cite:
 W.R. Pearson & D.J. Lipman PNAS (1988) 85:2444-2448

/tmp/fastaCAAsiaax5: 838 aa
 >LEX 157 SEQ ID NO:1 Taste Receptor
 vs  /tmp/fastaDAAtiaax5 library
searching /tmp/fastaDAAtiaax5 library

    841 residues in     1 sequences

FASTA (3.34 January 2000) function [optimized, BL50 matrix (15:-5)] ktup: 2
 join: 38, opt: 26, gap-pen: -12/ -2, width:  16
 Scan time:  0.050
The best scores are:                                                opt
gi|29294615|ref|NP_619642.2| sweet taste receptor  ( 841) 5708

>>gi|29294615|ref|NP_619642.2| sweet taste receptor T1r   (841 aa)
 initn: 4967 init1: 2709 opt: 5708
Smith-Waterman score: 5708;  99.524% identity in 841 aa overlap (1-838:1-841)

               10        20        30        40        50        60
LEX     MLLCTARLVGLQLLISCCWAFACHSTESSPDFTLPGDYLLAGLFPLHSGCLQVRHRPEVT
        ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
gi|292 MLLCTARLVGLQLLISCCWAFACHSTESSPDFTLPGDYLLAGLFPLHSGCLQVRHRPEVT
               10        20        30        40        50        60

               70        80        90       100       110
LEX     LCDRSCSFNEHGYHLFQAMRLGVEEINNSTALLPNITLGYQLYDVC-DSANVYATLRVLS
        ::::::::::::::::::::::::::::::::::::::::::::::: ::::::::::::
gi|292 LCDRSCSFNEHGYHLFQAMRLGVEEINNSTALLPNITLGYQLYDVCSDSANVYATLRVLS
               70        80        90       100       110       120

     120       130       140       150       160       170
LEX     LPGQHHIELQGDLLHYSPTVLAVIGPDSTNRAATTAALLSPFLVPMISYAASSETLSVKR
        ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
gi|292 LPGQHHIELQGDLLHYSPTVLAVIGPDSTNRAATTAALLSPFLVPMISYAASSETLSVKR
              130       140       150       160       170       180

     180       190       200       210       220       230
LEX     QYPSFLRTIPNDKYQVETMVLLLQKFGWTWISLVGSSDDYGQLGVQALENQATGQGICIA
        ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
gi|292 QYPSFLRTIPNDKYQVETMVLLLQKFGWTWISLVGSSDDYGQLGVQALENQATGQGICIA
              190       200       210       220       230       240

     240       250       260       270       280       290
LEX     FKDIMPFSAQVGDERMQCLMRHLAQAGATVVVVFSSRQLARVFFESVVLTNLTGKVWVAS
        ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
gi|292 FKDIMPFSAQVGDERMQCLMRHLAQAGATVVVVFSSRQLARVFFESVVLTNLTGKVWVAS
              250       260       270       280       290       300

     300       310       320       330       340       350
LEX     EAWALSRHITGVPGIQRIGMVLGVAIQKRAVPGLKAFEEAYARADKEAPRPCHKGSWCSS
        ::::::::::::::::::::::::::::::::::::::::::::::.:::::::::::::
gi|292 EAWALSRHITGVPGIQRIGMVLGVAIQKRAVPGLKAFEEAYARADKKAPRPCHKGSWCSS
              310       320       330       340       350       360

     360       370        380       390       400       410
LEX     NQLCRECQAFM-HTMPKLKAFSMSSAYNAYRAVYAVAHGLHQLLGCASGACSRGRVYPWQ
```

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       :::::::::: :::::::::::::::::::::::::::::::::::::::::::::::::
gi|292 NQLCRECQAFMAHTMPKLKAFSMSSAYNAYRAVYAVAHGLHQLLGCASGACSRGRVYPWQ
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      420       430       440       450       460       470
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       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
gi|292 LLEQIHKVHFLLHKDTVAFNDNRDPLSSYNIIAWDWNGPKWTFTVLGSSTWSPVQLNINE
              430       440       450       460       470       480

      480       490       500       510       520       530
LEX    TKIQWHGKDNQVPKSVCSSDCLEGHQRVVTGFHHCCFECVPCGAGTFLNKSDLYRCQPCG
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
gi|292 TKIQWHGKDNQVPKSVCSSDCLEGHQRVVTGFHHCCFECVPCGAGTFLNKSDLYRCQPCG
              490       500       510       520       530       540

      540       550       560       570       580       590
LEX    KEEWAPEGSQTCFPRTVVFLALREHTSWVLLAANTLLLLLLLGTAGLFAWHLDTPVVRSA
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
gi|292 KEEWAPEGSQTCFPRTVVFLALREHTSWVLLAANTLLLLLLLGTAGLFAWHLDTPVVRSA
              550       560       570       580       590       600

      600       610       620       630       640       650
LEX    GGRLCFLMLGSLAAGSGSLYGFFGEPTRPACLLRQALFALGFTIFLSCLTVRSFQLIIIF
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
gi|292 GGRLCFLMLGSLAAGSGSLYGFFGEPTRPACLLRQALFALGFTIFLSCLTVRSFQLIIIF
              610       620       630       640       650       660

      660       670       680       690       700       710
LEX    KFSTKVPTFYHAWVQNHGAGLFVMISSAAQLLICLTWLVVWTPLPAREYQRFPHLVMLEC
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
gi|292 KFSTKVPTFYHAWVQNHGAGLFVMISSAAQLLICLTWLVVWTPLPAREYQRFPHLVMLEC
              670       680       690       700       710       720

      720       730       740       750       760       770
LEX    TETNSLGFILAFLYNGLLSISAFACSYLGKDLPENYNEAK-VTFSLLFNFVSWIAFFTTA
       :::::::::::::::::::::::::::::::::::::::: ::::::::::::::::::
gi|292 TETNSLGFILAFLYNGLLSISAFACSYLGKDLPENYNEAKCVTFSLLFNFVSWIAFFTTA
              730       740       750       760       770       780

      780       790       800       810       820       830
LEX    SVYDGKYLPAANMMAGLSSLSSGFGGYFLPKCYVILCRPDLNSTEHFQASIQDYTRRCGS
       ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
gi|292 SVYDGKYLPAANMMAGLSSLSSGFGGYFLPKCYVILCRPDLNSTEHFQASIQDYTRRCGS
              790       800       810       820       830       840


LEX    T
       :
gi|292 T


838 residues in 1 query   sequences
841 residues in 1 library sequences
 Scomplib [version 3.3t05 March 30, 2000]
 start: Mon Nov 10 11:42:44 2003 done: Mon Nov 10 11:42:44 2003
 Scan time:  0.050 Display time:  0.983
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Function used was FASTA

☐ 1: NP_619642. sweet taste recep...[gi:29294615] BLink, Domains, Links

LOCUS NP_619642 841 aa linear PRI 05-OCT-2003
DEFINITION sweet taste receptor T1r isoform b; G protein-coupled receptor 70 [Homo sapiens].
ACCESSION NP_619642
VERSION NP_619642.2 GI:29294615
DBSOURCE REFSEQ: accession NM_138697.2
KEYWORDS .
SOURCE Homo sapiens (human)
ORGANISM Homo sapiens
Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
REFERENCE 1 (residues 1 to 841)
AUTHORS Li,X., Staszewski,L., Xu,H., Durick,K., Zoller,M. and Adler,E.
TITLE Human receptors for sweet and umami taste
JOURNAL Proc. Natl. Acad. Sci. U.S.A. 99 (7), 4692-4696 (2002)
MEDLINE 21927605
PUBMED 11917125
REFERENCE 2 (residues 1 to 841)
AUTHORS Nelson,G., Chandrashekar,J., Hoon,M.A., Feng,L., Zhao,G., Ryba,N.J. and Zuker,C.S.
TITLE An amino-acid taste receptor
JOURNAL Nature 416 (6877), 199-202 (2002)
MEDLINE 21891095
PUBMED 11894099
REMARK GeneRIF: sequence differences in T1R receptors within and between species (human and mouse) can significantly influence the selectivity and specificity of taste responses
REFERENCE 3 (residues 1 to 841)
AUTHORS Makalowska,I., Sood,R., Faruque,M.U., Hu,P., Robbins,C.M., Eddings,E.M., Mestre,J.D., Baxevanis,A.D. and Carpten,J.D.
TITLE Identification of six novel genes by experimental validation of GeneMachine predicted genes
JOURNAL Gene 284 (1-2), 203-213 (2002)
MEDLINE 21888635
PUBMED 11891061
COMMENT REVIEWED REFSEQ: This record has been curated by NCBI staff. The reference sequence was derived from AF387618.1 and BK000153.1.
On Mar 27, 2003 this sequence version replaced gi:20162558.

Summary: The protein encoded by this gene is a G protein-coupled receptor and is a component of the heterodimeric amino acid taste receptor T1R1+3. The T1R1+3 receptor responds to L-amino acids but not to D-enantiomers or other compounds. Most amino acids that are perceived as sweet activate T1R1+3, and this activation is strictly dependent on an intact T1R1+3 heterodimer. Multiple transcript variants encoding several different isoforms have been found for this gene.

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                     /map="1p36.23"
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                     wide range of receptors. This family also includes the
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                     /db_xref="CDD:pfam01094"
     Region          588..822
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                     glutamate family)"
                     /note="7tm_3"
                     /db_xref="CDD:pfam00003"
     CDS             1..841
                     /gene="TAS1R1"
                     /coded_by="NM_138697.2:1..2526"
                     /db_xref="GeneID:80835"
                     /db_xref="LocusID:80835"
                     /db_xref="MIM:606225"
ORIGIN
        1 mllctarlvg lqllisccwa fachstessp dftlpgdyll aglfplhsgc lqvrhrpevt
       61 lcdrscsfne hgyhlfqamr lgveeinnst allpnitlgy qlydvcsdsa nvyatlrvls
      121 lpgqhhielq gdllhysptv lavigpdstn raattaalls pflvpmisya assetlsvkr
      181 qypsflrtip ndkyqvetmv lllqkfgwtw islvgssddy gqlgvqalen qatgqgicia
      241 fkdimpfsaq vgdermqclm rhlaqagatv vvvfssrqla rvffesvvlt nltgkvwvas
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      361 nqlcrecqaf mahtmpklka fsmssaynay ravyavahgl hqllgcasga csrgrvypwq
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      481 tkiqwhgkdn qvpksvcssd cleghqrvvt gfhhccfecv pcgagtflnk sdlyrcqpcg
      541 keewapegsq tcfprtvvfl alrehtswvl laantllll llgtaglfaw hldtpvvrsa
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      721 tetnslgfil aflyngllsi safacsylgk dlpenyneak cvtfsllfnf vswiafftta
      781 svydgkylpa anmmaglssl ssgfggyflp kcyvilcrpd lnstehfqas iqdytrrcgs
      841 t
//
```

Human receptors for sweet and umami taste

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Communicated by Henry R. Bourne, University of California, San Francisco, CA, February 14, 2002 (received for review December 5, 2001)

The three members of the T1R class of taste-specific G protein-coupled receptors have been hypothesized to function in combination as heterodimeric sweet taste receptors. Here we show that human T1R2/T1R3 recognizes diverse natural and synthetic sweeteners. In contrast, human T1R1/T1R3 responds to the umami taste stimulus L-glutamate, and this response is enhanced by 5′-ribonucleotides, a hallmark of umami taste. The ligand specificities of rat T1R2/T1R3 and T1R1/T1R3 correspond to those of their human counterparts. These findings implicate the T1Rs in umami taste and suggest that sweet and umami taste receptors share a common subunit.

Large-scale sequencing of a subtracted cDNA library derived from rat taste tissue identified a new C-family G protein-coupled receptor, T1R1, that is expressed selectively in taste receptor cells; T1R1-based degenerate PCR led to the identification of a related taste-specific receptor, T1R2 (1). Recently, a third and possibly final member of the T1R family, T1R3, was identified in the human DNA databank (2–7). Tellingly, mouse T1R3 maps to a genomic interval containing *Sac*, a locus that influences sweet taste in the mouse (8, 9). Recent high-resolution genetic mapping and complementation studies have strengthened the connection between mouse T1R3 and *Sac* (2–7). Although T1R1 and T1R2 appear to be expressed in predominantly nonoverlapping regions of the tongue, they each are coexpressed with T1R3 (1, 3, 4, 6). These overlapping expression patterns and precedent from the structurally related heterodimeric γ-aminobutyric acid type B receptor (10–13) suggested that T1R1 and T1R2 may combine with T1R3 to form heterodimeric sweet taste receptors. Indeed, rat T1R2 has been shown recently to function in combination with T1R3 to recognize a subset of sweet taste stimuli, a finding that has been proposed to reflect the involvement of additional combinations of T1Rs in sweet taste (6). In this study we cloned and functionally expressed human and rat T1Rs. Human and rat T1R2/T1R3 recognized all sweet taste stimuli tested, and human and rat T1R1/T1R3 recognized umami taste stimuli. These findings suggest that different combinations of T1Rs function as heterodimeric sweet and umami taste receptors.

Material and Methods

T1R Cloning. Intronless human T1R expression constructs were generated in a pEAK10-derived vector (Edge Biosystems, Gaithersburg, MD) by a combination of cDNA-based and genomic DNA-based methods. To generate the full-length T1R1 expression construct, two 5′ coding exons identified in a cloned T1R1 interval (GenBank accession no. AL159177) were combined by PCR overlap and then joined to a 5′-truncated testis cDNA clone. The T1R2 expression construct was generated from a partially sequenced T1R2 genomic interval. Two missing T1R2 5′ introns were identified by screening shotgun libraries of the cloned genomic interval using probes derived from the corresponding rat coding sequence. Coding exons then were combined by PCR overlap to produce the full-length expression construct. The T1R3 expression construct was generated by PCR overlap from a sequenced T1R3 genomic interval (GenBank accession no. AL139287). Rat T1R3 was isolated from a taste-tissue-derived cDNA library by using a rat T1R3 exon fragment generated by human T1R3-based degenerate PCR.

$G_{\alpha 15}$ chimeras were generated in a pEAK10-derived vector by PCR with mutagenic primers. The five-residue C-terminal tail of $G_{\alpha 15}$, EINLL, was replaced with EYNLV ($G_{\alpha q}$ and $G_{\alpha 11}$), EFNLV ($G_{\alpha 14}$), QYELL ($G_{\alpha s}$ and $G_{\alpha olf}$), DCGLF ($G_{\alpha i1}$, $G_{\alpha i2}$, $G_{\alpha t1}$, $G_{\alpha t2}$, and $G_{\alpha gust}$), ECGLY ($G_{\alpha i3}$), GCGLY ($G_{\alpha o1}$ and $G_{\alpha o2}$), YIGLC ($G_{\alpha z}$), DIMLQ ($G_{\alpha 12}$), or QLMLQ ($G_{\alpha 13}$).

Taste Detection Thresholds. Detection threshold values were determined following the methods of Schiffman *et al.* (14) and averaged over three trials for four subjects.

T1R Functional Expression. HEK-293T and a HEK-293 derivative that stably expresses $G_{\alpha 15}$ (Aurora Biosciences, San Diego; ref. 15) were grown and maintained at 37°C in DMEM supplemented with 10% FBS and MEM nonessential amino acids (GIBCO/BRL); the medium for $G_{\alpha 15}$ cells also contained 3 $\mu g \cdot ml^{-1}$ blasticidin (GIBCO/BRL). For calcium-imaging experiments, cells first were seeded onto 24-well tissue-culture plates (≈100,000 cells per well) and transfected by using Mirus TransIt-293 (Panvera, Madison, WI). Transfection efficiencies, which were estimated by cotransfection with a red fluorescent protein expression vector, were typically ≈70%. To minimize glutamate-induced and glucose-induced desensitization, supplemented DMEM was replaced with low-glucose DMEM supplemented with GlutaMAX and 10% dialyzed FBS (GIBCO/BRL) ≈24 h after transfection. After an additional 24 h, cells were loaded with the calcium dye fluo-4 acetoxymethyl ester (Molecular Probes), 3 μM in Dulbecco's PBS buffer (DPBS, GIBCO/BRL), for 1.5 h at room temperature. After replacement with 250 μl of DPBS, stimulation was performed at room temperature by the addition of 200 μl of DPBS supplemented with taste stimuli. Calcium mobilization was monitored on an Axiovert S100 microscope equipped with an inverted ×10/0.5 long working distance plano fluor objective (Zeiss) and a cooled charge-coupled device camera (Princeton Instruments, Trenton, NJ). Fluorescence images were acquired at 480-nm excitation and 535-nm emission and analyzed with IMAGING WORKBENCH 4.0 software (Axon Instruments, Foster City, CA). T1R receptor activity was quantitated by counting the number of responding cells 30 sec after stimulus addition. Stimuli were tested at concentrations that do not elicit calcium responses from mock-transfected $G_{\alpha 15}$ cells. Compounds used (and typical concentrations) were: acesulfame K (2.5 mM), L-AP4 (10 mM), aspartame (2.5 mM), D-aspartate (50 mM), L-aspartate (50 mM), CMP (5 mM), cyclamate (5 mM), denatonium benzoate (5 mM), dulcin (0.1 mM), fructose (300 mM), galactose (300 mM), glucose (300 mM), D-glutamate (25 mM), L-glutamate (25 mM), L-glutamine (10 mM), glycine (250 mM), GMP (1 mM), L-histidine (10 mM), IMP (1 mM), lactose (250 mM), L-leucine (10 mM), L-lysine (10 mM), maltose (300 mM), monellin (0.01%), neotame (0.1 mM), perillartine (15 μM), L-proline (10 mM), quinine hydrochloride (0.25 mM), saccharin (1 mM), L-serine (10 mM), sodium chlo-

Data deposition: The sequences reported in this paper have been deposited in the GenBank database (accession nos.: human T1R1, BK000153; human T1R2, BK000151; human T1R3, BK000152; and rat T1R3, AF456324).

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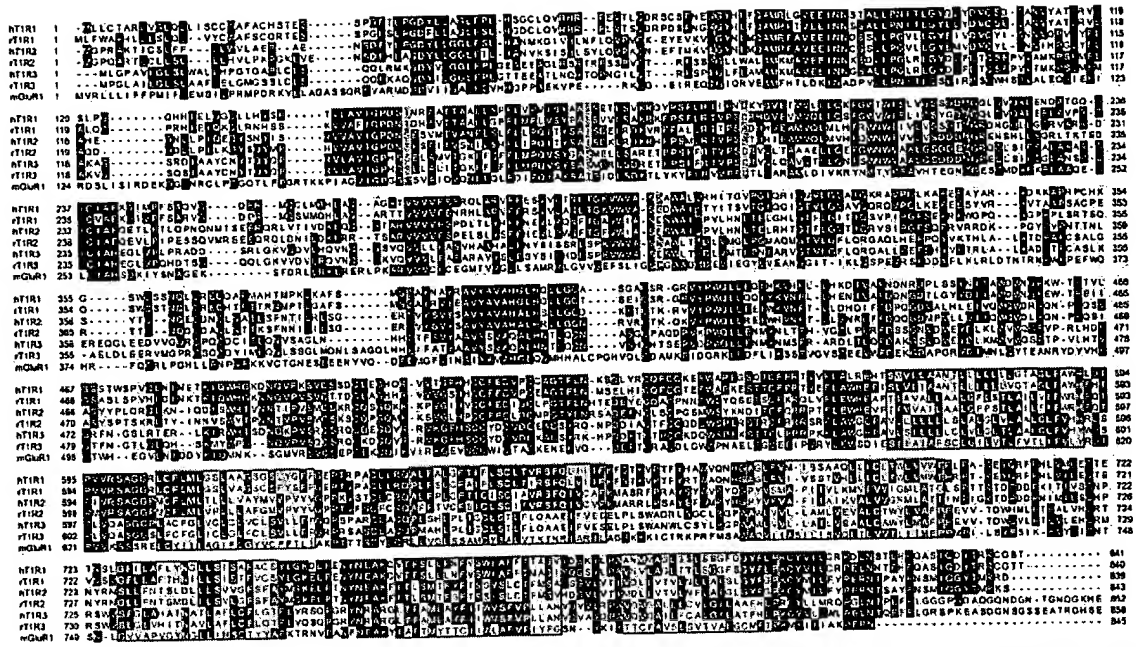

Fig. 1. Sequence alignment of human and rat T1Rs. T1R sequences determined in this study, human T1R1 (GenBank accession no. BK000153), T1R2 (accession no. BK000151), T1R3 (accession no. BK000152), and rat T1R3 (accession no. AF456324), are aligned here with previously described rat T1Rs (accession nos. AAD18069 and AAD18070) and the rat mGluR1 metabotropic glutamate receptor (accession no. P23385). The mGluR1 C terminus is not shown. Potential transmembrane segments are boxed in blue. mGluR1 ligand-binding residues are highlighted following the color scheme used in Fig. 3A.

ride (100 mM), sucralose (1 mM), sucrose (300 mM), thaumatin (0.01%), D-tryptophan (10 mM), L-tryptophan (10 mM), and L-tyrosine (5 mM). DPBS adjusted to pH 4.5 with acetic acid was used as a sour taste stimulus.

Human T1R2/T1R3 stable cell lines were generated by transfecting linearized pEAK10-derived T1R2 and pCDNA3.1/ZEO-derived (Invitrogen) T1R3 vectors into $G_{\alpha 15}$ cells. Cells were selected in 0.5 $\mu g \cdot ml^{-1}$ puromycin (Calbiochem) and 100 $\mu g \cdot ml^{-1}$ zeocin (Invitrogen) at 37°C in low-glucose DMEM supplemented with GlutaMAX/10% dialyzed FBS/3 $\mu g \cdot ml^{-1}$ blasticidin. Resistant colonies were expanded, and their responses to sweet taste stimuli were evaluated by fluorescence microscopy. For automated fluorometric imaging on VIPR-II instrumentation (Aurora Biosciences), T1R2/T1R3 stable cells first were seeded onto 96-well plates (≈15,000 cells per well). After 24 h, cells were loaded with the calcium dye fluo-3 acetoxymethyl ester (Molecular Probes), 5 μM in PBS, for 1 h at room temperature. After replacement with 70 μl of PBS, stimulation was performed at room temperature by the addition of 70 μl of PBS supplemented with taste stimuli. Fluorescence (480-nm excitation and 535-nm emission) responses from 20 to 30 sec after compound addition were averaged, corrected for background fluorescence measured before compound addition, and normalized to the response to the calcium ionophore ionomycin (1 μM, Calbiochem).

Results and Discussion

We cloned human and rat T1Rs for functional expression experiments (Fig. 1). The G protein or G proteins that couple to the T1Rs *in vivo* are not known. Consequently, we transiently transfected the human T1Rs into a HEK-293-derived cell line that stably expresses $G_{\alpha 15}$, a promiscuous phospholipase C-linked G protein (15, 16). Sucrose elicited transient intracellular calcium increases in $G_{\alpha 15}$ cells cotransfected with human T1R2 and T1R3 but not in cells transfected with T1R2 or T1R3 alone. T1R2/T1R3 activity was inhibited by the sweet taste inhibitor lactisole (17); this inhibition likely reflects antagonism at the T1R2/T1R3 receptor, because lactisole did not inhibit the $G_{\alpha 15}$-dependent activity of endogenous β_2-adrenergic receptor (Fig. 2*A*). In addition to sucrose, T1R2/T1R3 responded to all other sweet taste stimuli tested: the sugars fructose, galactose, glucose, lactose, and maltose; the amino acids glycine and D-tryptophan (but not its bitter enantiomer); the sweet proteins monellin and thaumatin; and the synthetic sweeteners acesulfame K, aspartame, cyclamate, dulcin, neotame, saccharin, and sucralose (Fig. 2*B*).

The weak responses of T1R2/T1R3-transfected cells precluded quantitating receptor activity by monitoring summated fluorescence changes over fields of calcium-dye-loaded cells as, for example, in Chandrashekar *et al.* (15). However, increased receptor activity in $G_{\alpha 15}$-based assays is reflected not only in increased magnitudes of the calcium responses of individual cells but also in increased numbers of responding cells. Therefore, we quantitated T1R receptor activity by counting the number of responding cells after stimulus addition. To validate this method, EC_{50} values were determined for several test receptors including the mGluR4 metabotropic glutamate receptor and mT2R5 cycloheximide receptor and found to correspond to published values (data not shown). The dose responses of T1R2/T1R3 were determined in this manner for several sweeteners, which activated T1R2/T1R3 at physiologically relevant concentrations with a rank order similar to human taste (Fig. 2*C*). Moreover, stable expression in $G_{\alpha 15}$ cells markedly increased T1R2/T1R3 activity and allowed us to quantitate receptor activity by monitoring summated calcium signals with automated fluorometric imaging. The increased activity of the T1R2/T1R3 stable cell line resulted in 2–5-fold left-shifted dose-response curves relative to cells transiently transfected with T1R2/T1R3 (Fig. 2*D*). This close correspondence further validates quantification of receptor activity by counting cells.

The response of human T1R2/T1R3 to all sweet taste stimuli tested contrasted with the limited response of rat

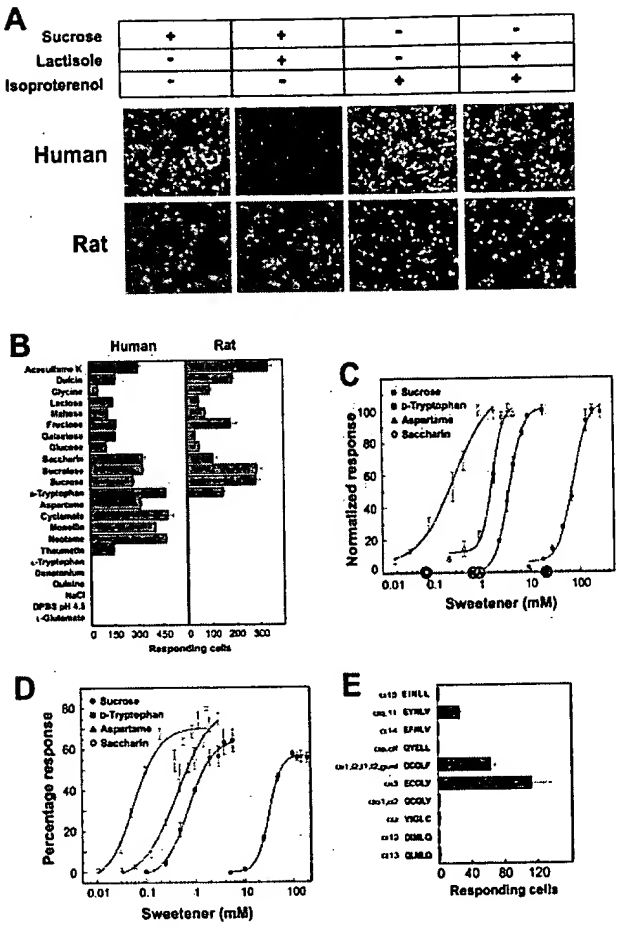

Fig. 2. Human and rat T1R2/T1R3 recognize sweet taste stimuli. (*A*) $G_{\alpha 15}$ cells transiently transfected with human T1R2 and T1R3 and HEK-293T cells transiently transfected with rat T1R2, T1R3, and $G_{\alpha 15/i1}$ were assayed for intracellular calcium increases in response to 300 mM sucrose in the presence (+) and absence (−) of 1.25 mM lactisole and to 10 nM isoproterenol in the presence and absence of 1.25 mM lactisole. Each imaged field shown contains ≈1,000 confluent cells. (*B*) The responses of human T1R2/T1R3 and rat T1R2/T1R3 were determined for sweet taste stimuli at the concentrations listed in *Materials and Methods*. (*C*) Human T1R2/T1R3 dose responses were determined for sucrose, D-tryptophan, aspartame, and saccharin. Dose responses were normalized to the maximal percentage of responding cells, which ranged from 10 to 40% for different sweeteners. Values represent the mean ± SE of four independent responses. The *x* axis circles represent average psychophysical detection threshold values for these four sweeteners. (*D*) The dose responses of $G_{\alpha 15}$ cells stably transfected with human T1R2 and T1R3 were determined for sucrose, D-tryptophan, aspartame, and saccharin. Responses are shown as the percentage of fluorescence values relative to fluorescence increases elicited by 1 μM ionomycin. Values represent the mean ± SE of four independent responses. (*E*) HEK-293T cells were transiently transfected with rat T1R2, rat T1R3, and each $G_{\alpha 15}$ chimera, and assayed for intracellular calcium increases in response to 75 mM sucrose. The five C-terminal residues of each $G_{\alpha 15}$ chimera are shown. The activities in *B* and *E* represent the mean ± SE number of responding cells for four imaged fields of ≈1,000 confluent cells.

T1R2/T1R3 determined previously by functional expression in heterologous cells and coupling to $G_{\alpha 16/z44}$ (a $G_{\alpha 16}$ variant containing the C-terminal 44 residues of $G_{\alpha z}$; refs. 6 and 18). To address this discrepancy, we transfected rat T1R2 and T1R3 into $G_{\alpha 15}$ cells and found that only high concentrations of a small number of sweet taste stimuli elicited detectable responses. These weak responses prompted us to investigate the possibility that rat T1R2/T1R3 responses to additional sweet taste stimuli might be obscured by deficiencies in the $G_{\alpha 15}$- and $G_{\alpha 16/z44}$-based assays such as poor expression of the rat T1Rs or inefficient G protein coupling. Several $G_{\alpha i}$-linked receptors have been shown to couple more efficiently to a $G_{\alpha q}$ variant containing the C-terminal five residues of $G_{\alpha i2}$ than to $G_{\alpha q}$ (19) and more efficiently to $G_{\alpha 16/z44}$ than to $G_{\alpha 16}$ (18). Accordingly, we reasoned that replacing the $G_{\alpha 15}$ C terminus with that of the G protein that couples to T1R2/T1R3 *in vivo* would improve coupling efficiency. Because the identity of this G protein is not known, we generated a panel of $G_{\alpha 15}$ chimeras in which the five-residue C-terminal tail of $G_{\alpha 15}$ was replaced by the tails of all other G proteins. The response of rat T1R2/T1R3-transfected HEK-293T cells to sucrose was enhanced in the presence of chimeras containing the C termini of $G_{\alpha i}$-related G proteins or a chimera containing the C terminus of $G_{\alpha q}$ (Fig. 2*E*). Transient cotransfection of a $G_{\alpha 15}$ chimera containing the C-terminal tail of $G_{\alpha i1}$, $G_{\alpha 15/i1}$, with rat T1R2 and T1R3 into HEK-293T cells revealed responses to all sweet taste stimuli tested (except for compounds such as aspartame and monellin that taste sweet to humans but are not palatable to rodents; Fig. 2*B*; refs. 20 and 21). Lactisole, which does not inhibit sweet taste in rats (17), did not inhibit rat T1R2/T1R3 activity (Fig. 2*A*).

The inclusive ligand specificity of human and rat T1R2/T1R3 suggested that T1R1/T1R3 mediates a different taste modality. We speculated that T1R1/T1R3 might function as an umami taste receptor because key ligand-binding residues of the mGluR1 metabotropic glutamate receptor are conserved in T1R1 (Figs. 1 and 3*A*; ref. 22). Indeed, L-glutamate elicited transient intracellular calcium increases in $G_{\alpha 15}$ cells cotransfected with human T1R1 and T1R3 but not in cells transfected with T1R1 or T1R3 alone (Fig. 3 *B* and *C*).

Robust synergism between L-glutamate and the 5′ ribonucleotides IMP and GMP is a hallmark of umami taste (23). IMP or GMP alone did not activate human T1R1/T1R3, but these 5′ ribonucleotides potentiated the T1R1/T1R3 response to L-glutamate; CMP, which does not enhance umami taste, had no effect (Fig. 3 *B* and *D*). In the presence of 0.2 mM IMP, the EC_{50} of T1R1/T1R3 for L-glutamate was shifted 30-fold; this dramatic effect on T1R1/T1R3 activity was similar to the effect of these compounds on umami taste (Fig. 3*C*). The effect of IMP on T1R1/T1R3 was saturable (Fig. 3*E*) and selective; human T1R2/T1R3 was not activated by L-glutamate in the presence of IMP, and IMP did not enhance the response of human T1R2/T1R3 to sweet taste stimuli (Fig. 3*F*). In contrast to L-glutamate, T1R1/T1R3-transfected cells did not respond to the weak umami taste stimuli L-aspartate and L-AP4 (23). In the presence of IMP and GMP, however, these compounds activated T1R1/T1R3 at physiologically relevant concentrations (Fig. 3 *D* and *G*). The response of T1R1/T1R3 was selective for umami taste stimuli; T1R1/T1R3 did not respond to sweet taste stimuli, amino acids such as D-glutamate and D-aspartate, or binary mixtures of these compounds with IMP (Fig. 3*H*).

As in human taste, IMP enhances L-glutamate taste in rodents, and rodent taste tests suggest that L-aspartate and L-AP4 mimic L-glutamate (24–26). Cotransfection of $G_{\alpha 15/i1}$ with rat T1R1 and T1R3 into HEK-293T cells revealed responses to binary mixtures of IMP with L-glutamate and L-aspartate but not L-AP4; responses to L-glutamate and L-aspartate were observed also in the presence of GMP but not CMP (Fig. 3*I*). L-Glutamate and L-aspartate in the presence of 2.5 mM IMP activated rat T1R1/T1R3 with similar potency (Fig. 3*J*). Rat T1R1/T1R3 did not respond to sweet taste stimuli such as sucrose and D-tryptophan, D-glutamate, D-aspartate, or binary mixtures of these compounds with IMP (data not shown).

In summary, our findings suggest that different combinations of T1Rs function as sweet and umami taste receptors. Intriguingly,

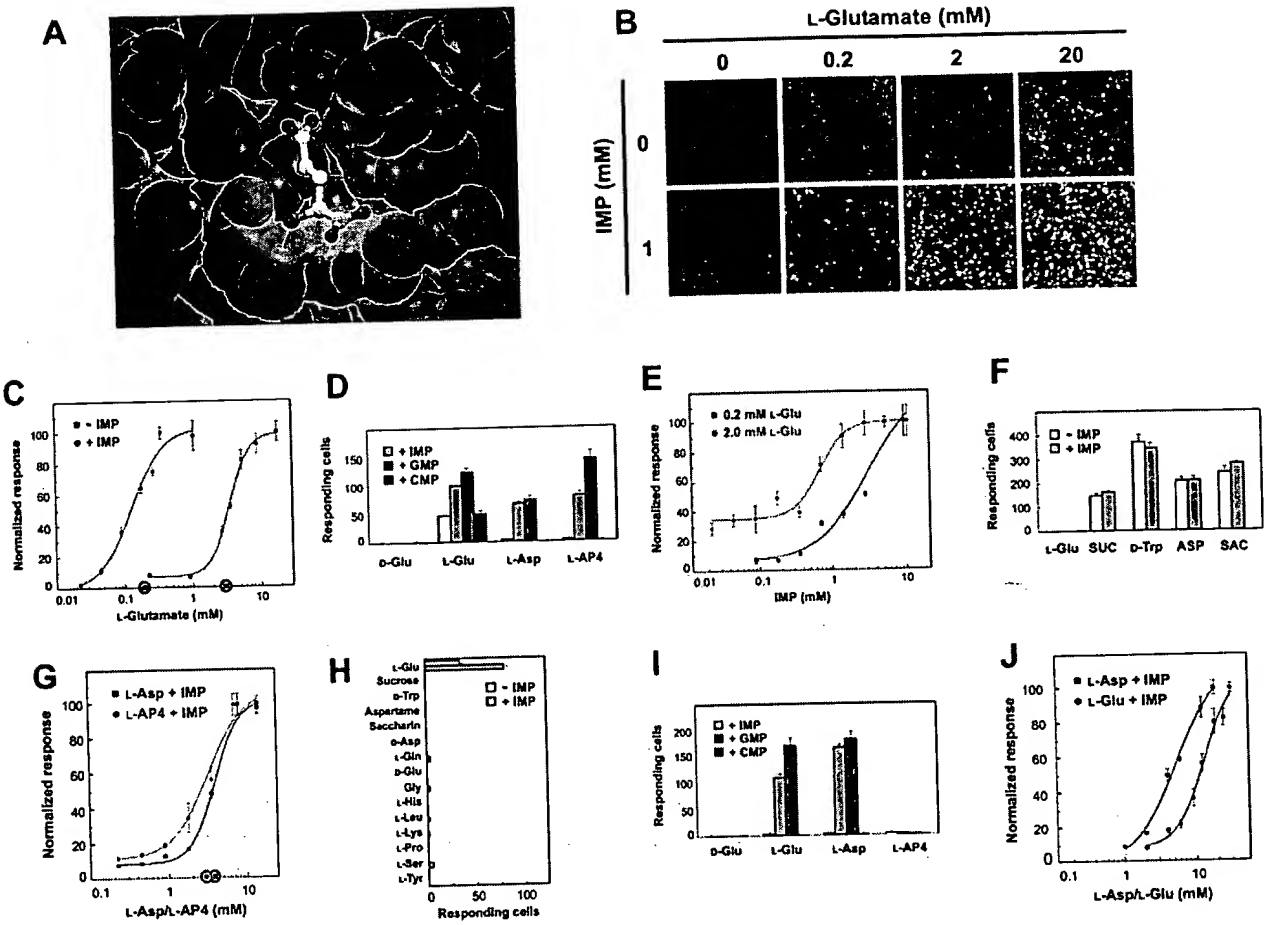

Fig. 3. Human and rat T1R1/T1R3 recognize umami taste stimuli. (*A*) mGluR1 residues (PDB entry no. 1EWK) that contact the L-glutamate side chain carboxylate are shown in red, and residues that contact the L-glutamate α-amino acid moiety are shown in green. (*B*) $G_{\alpha 15}$ cells transiently transfected with human T1R1 and T1R3 were assayed for intracellular calcium increases in response to increasing concentrations of L-glutamate in the presence or absence of 1 mM IMP. Each imaged field shown contains ≈1,000 confluent cells. (*C*) Human T1R1/T1R3 dose responses were determined for L-glutamate in the presence and absence of 0.2 mM IMP. Dose responses were normalized to the maximal percentage of responding cells, which was ≈5% for L-glutamate and ≈10% for L-glutamate plus IMP. Values represent the mean ± SE of four independent responses. The *x* axis circles represent average psychophysical detection threshold values for L-glutamate in the presence and absence of 0.2 mM IMP. (*D*) Human T1R1/T1R3 responses to 25 mM D-glutamate, 25 mM L-glutamate, 25 mM L-aspartate, 25 mM L-AP4, and binary mixtures of these compounds with 2.5 mM IMP, 2.5 mM GMP, or 2.5 mM CMP were determined. (*E*) Human T1R1/T1R3 dose responses were determined for IMP in the presence of 0.2 and 2 mM L-glutamate and normalized to the maximal percentages of responding cells, which were ≈10%. Values represent the mean ± SE of four independent responses. (*F*) Human T1R2/T1R3 responses to 25 mM L-glutamate, 100 mM sucrose (SUC), 2.5 mM D-tryptophan, 1.5 mM aspartame (ASP), and 0.4 mM saccharin (SAC) were determined in the presence and absence of 2.5 mM IMP. (*G*) Human T1R1/T1R3 dose responses were determined for L-aspartate and L-AP4 in the presence of 0.2 mM IMP. Dose responses were normalized to the maximal percentage of responding cells, which was ≈5% for L-aspartate plus IMP and ≈10% for L-AP4 plus IMP. Values represent the mean ± SE of four independent responses. The *x* axis circles represent average psychophysical detection threshold values for L-aspartate plus 0.2 mM IMP and L-AP4 plus 0.2 mM IMP. (*H*) Human T1R1/T1R3 responses to sucrose (100 mM), D-tryptophan (20 mM), aspartame (2 mM), saccharin (1 mM), L-tyrosine (5 mM), and D-glutamate, L-glutamine, D-aspartate, glycine, L-histidine, L-leucine, L-lysine, L-proline, and L-serine (each at 10 mM) were determined in the presence and absence of 1 mM IMP. (*I*) HEK-293T cells were transiently transfected with rat T1R1, rat T1R3, and $G_{\alpha 15/i1}$ and assayed for increases in intracellular calcium in response to 25 mM D-glutamate, 25 mM L-glutamate, 25 mM L-aspartate, 25 mM L-AP4, and binary mixtures with 2.5 mM IMP, 2.5 mM GMP, or 2.5 mM CMP. (*J*) Rat T1R1/T1R3 dose responses were determined for L-aspartate and L-glutamate in the presence of 0.2 mM IMP. Dose responses were normalized to the maximal percentage of responding cells, which was ≈10% for L-aspartate plus IMP and ≈15% for L-glutamate plus IMP. Values represent the mean ± SE of four independent responses. The activities in *D*, *F*, *H*, and *I* represent the mean ± SE number of responding cells for four imaged fields of ≈1,000 confluent cells.

human and rat T1R2/T1R3 were activated by all sweet taste stimuli tested. T1R2/T1R3 therefore may be the only sweet taste receptor. Analogously, the finding that all umami taste stimuli tested acted in combination with 5′ ribonucleotides at human T1R1/T1R3 raises the possibility that T1R1/T1R3 is the only umami taste receptor. Discrepancies between T1R1/T1R3 activity and umami taste (such as the lack of response of rat T1R1/T1R3-transfected cells to L-AP4) may reflect deficiencies in the $G_{\alpha 15}$-based assay or the involvement in umami taste of other receptors such as two previously identified candidate umami taste receptors (27, 28). Evaluation of these hypotheses awaits further experimentation such as characterization of T1R-null mice.

Preliminary analysis of T1R surface expression with epitope-tagged receptors revealed that the T1Rs are predominantly localized intracellularly (L.S., unpublished data). Such negligible amounts of surface receptors, although evidently sufficient for activity, confound coimmunoprecipitation experiments with whole-cell lysates and preclude biochemical analysis of T1R

interactions on the surface of heterologous cells. However, the hypothesis that mammalian T1Rs function as heterodimeric sweet and umami taste receptors is supported by functional codependence, coincident expression in taste receptor cells (3, 4, 6), and precedent from the structurally related heterodimeric γ-aminobutyric acid type B receptor (10–13). The presence of three T1R1-like and two T1R3-like genes in the pufferfish genome suggests that the emergence of heterodimeric C-family chemosensory receptors preceded the emergence of terrestrial animals.

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Peptide-Binding G Protein-Coupled Receptors: New Opportunities for Drug Design

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Abstract: Over the last decades distinct members of the G Protein-Coupled Receptor (GPCR) family emerged as prominent drug targets within pharmaceutical research, since approximately 60 % of marketed prescription drugs act by selectively addressing representatives of that class of transmembrane signal transduction systems. It is noteworthy that the majority of GPCR-targeted drugs elicit their biological activity by selective agonism or antagonism of biogenic monoamine receptors, while the development status of peptide-binding GPCR-adressing compounds is still in its infancy.

Exemplified on selected medicinal chemistry projects, this review will focus on the opportunities of therapeutic intervention into a broad spectrum of disease processes through agonizing or antagonizing the functions of peptide-binding GPCRs. In this context, a brief overview of GPCR-mediated signal transduction pathways will be given in order to emphasize the biomedical relevance of a controlled modulation of receptor function. Modern trends on lead finding and optimization strategies for peptide-binding GPCR-targeted low-molecular weight compounds will be highlighted on the basis of current research programs conducted in the areas of angiotensin II, endothelin, bradykinin, neurokinin, neuropeptide Y, LHRH, C5a antagonists, and somatostatin agonists, respectively. Special emphasis will be laid on the elaboration and utilization of structural rationales on the potential drug candidates, thus facilitating more detailed insights into the underlying molecular recognition event.

INTRODUCTION

Current pharmaceutical research is going through a period of unprecedented change, since new revolutionizing techniques have been successfully implemented into the pharmaceutical discovery process. At the same time, pharmaceutical industry feels growing pressure to release more new chemical entities (NCEs) that evolve as highly selective drugs targeting therapeutic areas of unmet medical need and address novel mechanisms of action. These attributes clearly define an ideal set of preconditions for positioning a candidate with block buster potential onto the drug market [1-3]. The conceptual combination of automated combinatorial chemistry, multiple parallel synthesis with high-throughput screening has dramatically altered the process of lead finding in medicinal chemistry in that vast numbers of low molecular weight compounds can rapidly be screened against biological target systems [4]. This progress in medicinal chemistry is paralleled on the side of target identification and validation with the maturation of genomics, proteomics, and bioinformatics in pharmaceutical research [5]. Taken together, these novel methodologies are expected to facilitate and accelerate the overall drug discovery process significantly.

However, the judicious choice of a disease relevant target is still one of the most crucial steps in initiating a drug discovery project, both in terms of novelty and uniqueness of the underlying therapeutic principle, as well as the competitor situation [2].

In this context, the superfamily of transmembrane G protein-coupled receptors (GPCRs) emerged as the most prominent class of qualified drug targets for pharmaceutical research and biomedical application [6]. Approximately 60% of all commercially available drugs work by selective modulation of distinct members of this target family [7]. Even though an estimated number of 1000 to 2000 GPCRs is expected to exist in the human genome [8], current GPCR-targeted therapeutic principles exploit a surprisingly small fraction of the GPCR family known today. A strong bias exists among the GPCR-targeted drugs in favour of the subclass of biogenic monoamine-stimulated GPCRs, i.e. the classical neurotransmitter-binding receptors [9,10].

This review will focus on the opportunity to further expand the spectrum of drug-targeted GPCRs onto the huge subclass of peptide-binding representatives of that target family. After a brief introduction on the basic principles of receptor structure and function, the chemically diverse set of endogenous ligands will be discussed with the aim to emphasize the relevance of peptide-binding GPCRs for modern drug discovery.

The lead identification and optimization attempts discussed in this contribution are restricted on projects that are aimed to identify peptidomimetic or non-peptide agonists or antagonists. Numerous pharmaceutical research efforts conducted over the last two decades have clearly proven the

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relevance of an early pharmacokinetic profiling. Consequently, satisfactory metabolic stability and oral bioavailability demand a transfer of the peptide-encoded biological and structural information onto non-peptide, drug-like scaffolds in order to achieve the desired goal [11-13].

Classical attempts towards drugs selectively addressing peptide-binding GPCRs will be exemplified on the angiotensin II and endothelin receptor antagonists. In both areas, leads were identified by screening programs and further optimized by classical medicinal chemistry approaches to yield clinical candidates, some of which already entered the market. The classical approach of optimizing screening hits will further be introduced with medicinal chemistry programs aimed to identify active compounds for a modulation of the bradykinin, neurokinin, and NPY (neuropeptide Y) receptors. Since the area of peptide-binding GPCR compounds is still in its infancy, especially when compared to the situation of biogenic amine-binding receptor drugs, the actual state of the majority of projects discussed in this review is still in the preclinical or in early clinical phases. Apart from random lead finding attempts, structural rationales are more frequently used in recent times, precedented by studies on somatostatin, bradykinin, neurokinin, LHRH (luteinizing hormone-releasing hormone), and anaphylatoxin C5a receptor agonists and antagonists that will be discussed briefly. Structural rationales were mainly derived from an educated guess on the bioactive conformation of the endogenous peptide or protein ligand, thus offering the opportunity to follow an indirect drug design approach.

GPCR SUPERFAMILY

G protein-coupled receptors constitute the largest receptor family known today [8]. According to an analysis of the *C. elegans* genome [14], approximately 5% of the 19100 nematode genes encode GPCRs with a family distribution profile that is reminiscent to that of mammalian GPCR genes. Extrapolation of these findings would suggest that up to 5000 distinct GPCR-encoding genes exist within the human genome (5% of an estimated 100000 genes). Currently, more than 800 distinct members of the GPCR superfamily have been cloned from various species, ranging from fungi over plants, yeast, slime mould, protozoa, metazoa to humans. Apart from the sensory olfactory receptors, approximately 150 human GPCRs have been cloned for which also the endogenous ligands have been identified. Further, more than 100 GPCRs are known with unidentified ligands and unknown physiological relevance, so called orphan GPCRs, which undoubtedly represent a rich source of disease-relevant drug targets for future biomedical research [15-17].

Structure and Function of GPCRs

GPCRs belong to the class of integral plasma membrane proteins and share a common receptor protein topology throughout the entire family. The structure paradigm is a seven helix bundle that spans the cell membrane in an almost perpendicular orientation, thereby establishing a functional link between the exterior and the cytoplasm of the

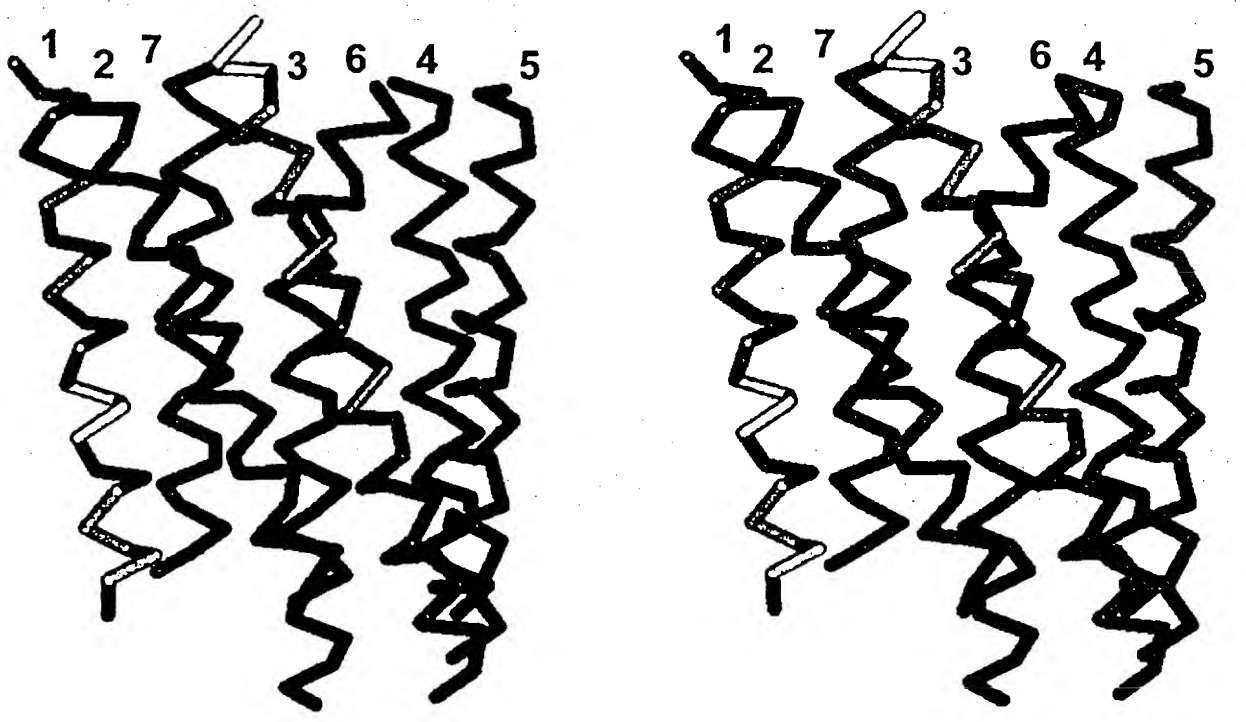


Fig. (1). Side-by-side stereo presentation of the Cα trace model of rhodopsin derived from various biophysical and bioinformatics studies. The helix bundle is shown in a sideview, the extracellular compartment being on the top. For details see references [22-31].

cell [6,18-20]. The seven transmembrane sequence stretches can be identified by hydrophobicity analyses since they exhibit an increased hydrophobic signature in a corresponding hydrophobicity profile. From numerous biophysical and biochemical studies it is now general accepted that GPCRs intercalate into the cell membrane with their *N*-terminus in the extracellular compartment, while the *C*-terminus reaches into the cytoplasm of the cell. The seven transmembrane helices (7TM domain) that constitute the central core domain of all GPCRs, are sequentially connected by extracellular and intracellular loops. Apart from variations in the primary structure, GPCRs differ in length of these loops, as well as in length and function of both *N*- and *C*-termini. The ACTH (adrenocorticotropic hormone) receptor is one of the smallest GPCRs known with 297 residues. Biogenic monoamine receptor sequences cover a size from approximately 350 to 600 residues, peptide receptor sequences are found between 400 and 750 residues, while the mGluRs (metabotropic glutamate receptor) mark the upper boundary consisting of roughly 1200 amino acid residues [21].

Even though no high-resolution structure of any pharmaceutical relevant member of the GPCR superfamily has been determined by e.g. x-ray crystallography, low resolution models derived from electron cryo-microscopy and electron diffraction of bovine, frog and squid rhodopsin reveal a detailed picture of the insertion mode of each helix within the context of the transmembrane helix bundle domain (Fig. (1)) [22-31].

From a functional point of view, GPCRs share a common property in that they work as transmembrane transducer systems by transferring an extracellular message across the cell membrane, thus allowing the affected tissue to respond to a broad range of signalling molecules [32-35]. Upon extracellular binding of the molecular stimulus, the central core domain (7TM domain) is believed to undergo a conformational change, thereby transmitting the extracellular binding event into the cytoplasm (Fig. (2)). The binding of a receptor agonist leads to an intracellular interaction of the receptor protein with its cognate heterotrimeric GDP-bound G protein. The agonist-promoted conformational change of the receptor protein followed by the cytoplasmic G protein-coupling initiates the activation of intracellular effector systems by the G protein cycle (Fig. (2)). The coupling event catalyzes the exchange of GDP against GTP and the dissociation of the GTP-bound α subunit from the βγ heterodimer. Depending on the very nature of the G protein α subtype, different effector systems such as enzymes (e.g. adenylyl cyclase, phospholipase C) or ion channels are functionally modulated, which substantially amplifies the production of second messengers. The effector activation event is accompanied by a GTPase activity of the α subunit releasing inorganic phosphate. The GDP-bound form converts the α subunit to exhibit high affinity for the βγ heterodimer, finally forming the GDP-bound heterotrimeric G protein again. The modulated concentration of second messengers elicits phosphorylation cascades across the cytoplasm to the nucleus, eventually activating the final physiological response of a cell to the original extracellular stimulus. Even though this functional paradigm accounts for all known GPCRs, this obvious convergence after the ligand binding event is diversified by the selective activation of only distinct types of G proteins from which e.g. numerous different G_α subunits are known (Fig. (2)) [32-35].

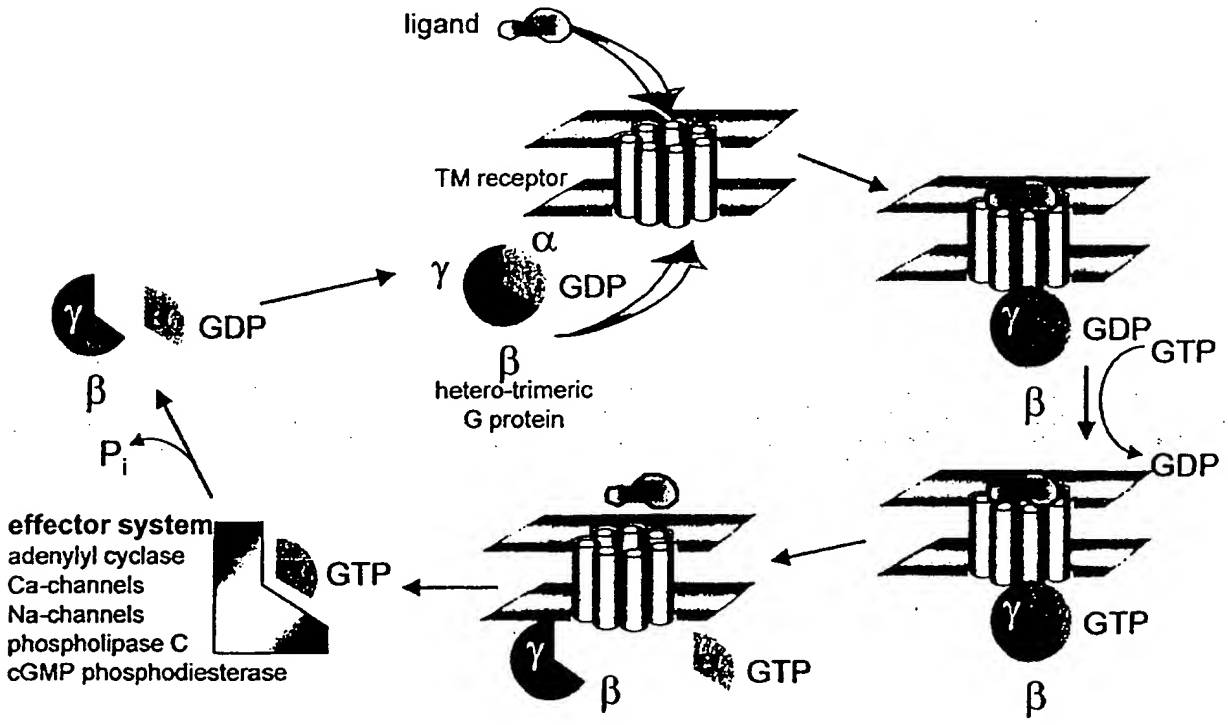


Fig. (2). Schematic representation of the ligand-GPCR interaction mediated G protein cycle.

In order to fully characterize the mechanism of action of GPCRs, a thermodynamic "eight-state-model" has been developed as a mechanistic hypothesis describing the macroscopic properties of transitions among distinct conformational states (Fig. (3)) [36]. The simplest way to describe the ligand-induced receptor activation event is a ternary complex model (A) that defines two distinct affinity states of the receptor for agonist binding, notably the free receptor (*Rec*) and the G protein-bound form (*G•Rec*) (Fig. (3)A). Agonists would display high affinity to the G protein-associated receptor, while antagonists would exhibit only low-affinity towards the complex. With the discovery that GPCRs can activate G proteins in the absence of any agonist, the simple ternary complex model required an extension. To account for the existence of such constitutively active GPCRs, a receptor activation step in the unliganded form was introduced (Fig. (3)B). This receptor isomerization hypothesis resulted in a "six-state-model" in which the activated receptor (*Rec**) is capable of signalling in both the G protein-associated form (*G•Rec**), and in the ternary complex (*G•Rec*•Lig*). The problem with that receptor activation-extended ternary complex model is that the G protein only binds to the receptor in its activated form *Rec**. Experimental evidence clearly suggests that G proteins do also bind to the resting state (*Rec*) without subsequent G protein activation. To account for these findings and to refer to the microscopic reversibility principle of thermodynamics, an "eight-state-model" was proposed in which the receptor protein can undergo three distinct processes, namely (i) ligand binding, (ii) receptor isomerization, and (iii) G protein binding (Fig. (3)C). Agonists can bind to four different receptor states clearly favouring the activated states generated either by receptor isomerization or by G protein association. Inverse agonists would prefer to bind the non-activated groundstate (*Rec*), while partial agonists show affinity to both receptor states but still cause receptor activation. In the thermodynamic "eight-state-model" an antagonist would just block the interconversion of different states rather than preferably bind to distinct states (Fig. (3)) [36].

In order to address phenomena such as isosteric or allosteric antagonism, structural models with atomic resolution are mandatory that are actually frequently used for both rationalizing structure-activity relationships of low molecular weight agonists and antagonists, as well as understanding the results from site-directed mutagenesis experiments. A detailed discussion of the actual status of experimentally derived, and molecular modeling derived GPCR structures [37] is beyond the scope of this review, since this contribution is mainly aimed to introduce the currently applied technologies to identify compounds selectively modulating peptide-binding GPCRs.

GPCR Classification

Exhaustive sequence analysis revealed three major homology families for the mammalian GPCRs, notably the family 1 or rho-family (prototype: rhodopsin), the family 2 or scr-family (prototype: secretin receptor), and the family 3 or mGluR family (prototype: metabotropic glutamate receptors) receptors (Fig. (4)) [32-35]. Family 1 receptors are divided into further subfamilies according to the size and

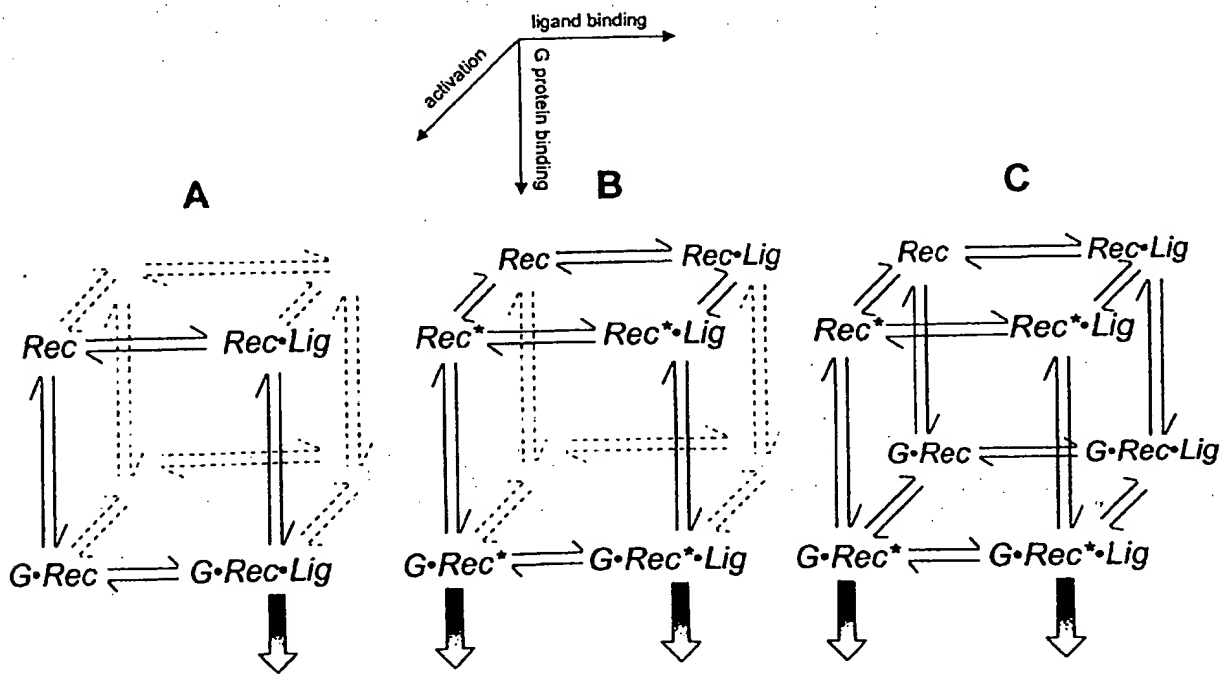


Fig (3). Mechanistic characterization of GPCR activation; A: "four-state" model; B: "six-state" model; C: "eight-state" model. Rec: receptor protein; Rec*: activated receptor protein; G: G protein; Lig: ligand molecule; filled arrows mark complexes capable of signalling (for details see text).

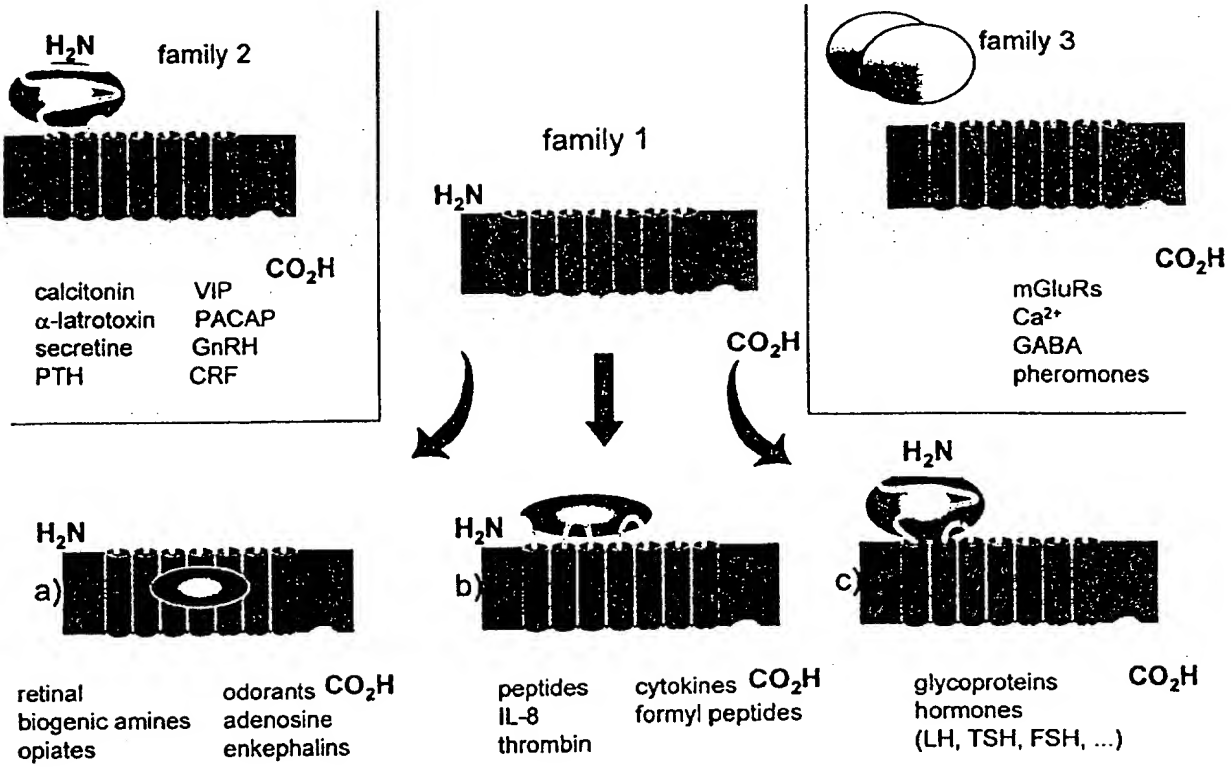


Fig. (4). Sequence homology-derived classification of GPCRs. Each GPCR family is characterized by a common ligand binding mode.

chemical nature of their corresponding agonists, as well as the mode of ligand binding. Family 1a accommodates the β-adrenoceptor-type receptors that are activated by small ligands such as biogenic monoamines, opiates, nucleotides, and small peptides, that comparably bind to a transmembrane cavity formed by helices 3, 4, 5, and 6. Family 1b is composed of receptors stimulated by oligopeptides and proteins such as IL-8 (interleukin-8), cytokines, and thrombin. The ligand binding epitope is located in the extracellular loop region. Family 1c receptors recognize glycoprotein hormones such as LH (luteinizing hormone), TSH (thyroid-stimulating hormone), and FSH (follicle-stimulating hormone) while their ligand binding site is centred in a large extracellular *N*-terminal domain (Fig. (4)).

Family 2 receptors are distinct from rho-family receptors in that they bind large peptides like glucagon, secretin, PTH (parathyroid hormone), VIP (vasointestinal peptide), or CRF (corticotropin-releasing factor). Comparable to family 1c receptors, the secretin family utilizes a large *N*-terminal domain for ligand binding. Family 3 receptors are unique since they possess a large extracellular *N*-terminal domain of several hundred residues that constitutes the binding site for smallish ligands such as a single divalent Ca^{2+} cation, glutamate, GABA (γ-amino butyric acid), and pheromones (Fig. (4)).

On the light of this classification, peptide-binding receptors are not structurally homogenous since they belong to family 1 and 2. Consequently, correlation of sequence homology with ligand similarity remains questionable which is also reflected by the mutual different binding modes of peptidic and non-peptidic agonists and antagonists.

Ligand Variety

GPCRs are stimulated by an amazingly large number of agonists covering a broad range of chemical diversity. Ligands are as small as divalent cations, biogenic monoamines such as acetylcholine or serotonin, fragrances and taste molecules such as aspartam or limonen, single amino acids such as glutamate or GABA, or nucleotide analogues such as adenosine. Medium-sized ligands range from cannabinoids over prostaglandines to small oligopeptides such as enkephalins, angiotensin II, bradykinin, somatostatin, and tachykinins. Larger oligopeptides and globular proteins constitute the family of macromolecular ligands including e.g. neuropeptide Y, C5a anaphylatoxin, interleukin-8, or chemokines. Even proteolytic enzymes such as thrombin, which activates its receptor by cleaving off an *N*-terminal peptide, selectively bind to distinct members of the GPCR superfamily. Apart from their important role in sensory perception including

vision, smell, and taste, GPCRs are obviously optimized by Nature for recognition and transduction of messages from different compound classes, i.e. nucleosides, lipid mediators, neurotransmitter, peptides, and proteins [6,18,38].

In this context, it is interesting to note that the majority of GPCR-targeted therapeutic principles exploit only a single compound class, notably the neurotransmitters. When the number of currently identified neurotransmitter receptors is compared with the number of disease-relevant peptide-binding GPCRs, an obvious imbalance becomes apparent in that only a small number of peptide-binding GPCRs is targeted by established therapies. Agonism and antagonism of e.g. α and β adrenoceptors, dopamine, histamine, serotonin, or muscarinic acetylcholine receptors are well established therapeutic principles for numerous "best-selling" drugs covering virtually all therapeutic areas, including gastrointestinal, cardiovascular, and CNS indications. In contrast, only two peptide-binding GPCR families are addressed by marketed non-peptide drugs, namely the opioid receptors and the angiotensin II receptor. However, the importance of peptide- and protein-binding GPCRs for drug discovery continues to be manifested by the fact that across current pharmaceutical research, especially in industry, numerous projects are pursued to identify leads that, upon optimizations fulfil all pharmacodynamic and pharmacokinetic demands required for clinical applicability (Table 1).

CLASSICAL LEAD FINDING AND DRUG DEVELOPMENT

Currently applied drug design and discovery approaches are typically classified as rational or random, depending on whether or not structural rationales are employed. The area of GPCR agonists and antagonists research is mainly driven by screening approaches in which large numbers of randomly selected chemical entities are tested in high-throughput screens. These shotgun procedures provide a practical means for identifying new leads for a particular receptor. In the following, this classical approach for GPCR-targeted drug discovery will be exemplified with prototype studies conducted on the angiotensin II, endothelin, bradykinin, neurokinin, and NPY receptors, respectively.

Table 1. Selection of endogenous Peptides that Exert their Biological Activity by Selective Activation of a GPCR

GPCR	code	native ligand (peptide/protein)	nature of the ligand
angiotensin receptors	AT_1, AT_2	angiotensin II (AII)	Asp-Arg-Val-Tyr-Ile-His-Pro-Phe
bombesin receptors	BB1 - BB4	bombesin, neuromedin B, gastrin-releasing peptide	14 aa peptide amide
bradykinin receptors	B_1, B_2	bradykinin (BK)	Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg
C3a receptor	C3aR	C3a anaphylatoxin	protein
C5a receptor	C5aR	C5a anaphylatoxin	protein
CC chemokine receptors	CCR1 - CCR9	chemokines	proteins
CXC chemokine receptors	CXCR1 - CXCR5	chemokines	proteins
cholecystokinin/gastrin receptors	CCK_A, CCK_B	cholecystokinin (CCK), gastrin	33 aa peptide amide, 17 aa peptide amide
endothelin receptors	ET_A, ET_B	endothelin-1 (ET-1), ET-2, ET-3	21 aa peptides
alpha factor pheromone receptor	STE2, STE3	fungal mating pheromones	13 aa peptide
fMet-Leu-Phe receptor	fMLP-R	Formylpeptide (fMLP)	FMet-Leu-Phe
galanin	GAL1, gal2, gal3	galanin	30 aa peptide
melanocortin receptors & ACTH receptor	MC_1, MC_3, MC_4, MC_5 MC_2 = ACTH receptor	melanocortin (MSH) adrenocorticotropic hormone (ACTH), corticotropin	39 aa peptide
neuropeptide Y receptor	Y_1 - Y_6	neuropeptide Y (NPY), peptide YY (PYY), pancreatic polypeptide (PP)	36 aa peptide amide (NPY)
neurotensin receptor	NTS1, nts2	neurotensin	13 aa peptide
opioid receptors	δ	[Met]-enkephalin, [Leu]-enkephalin	Tyr-Gly-Gly-Phe-Met/Leu
	κ	dynorphin A	17 aa peptide
	μ	β-endorphin, Lipotropin C fragment	31 aa peptide
nociceptin receptor	ORL1	nociceptin, orphanin FQ	17 aa peptide
somatostatin receptors	sst_1 - sst_5	somatostatin	cyclic14 aa peptide
tachykinin receptors	NK_1	substance P	11 aa peptide
	NK_2	neurokinin A (NKA), substance K, neuromedin L	His-Lys-Thr-Asp-Ser-Phe-Val-Gly-Leu-Met-NH_2
	NK_3	neurokinin B (NKB), neuromedin K	Asp-Met-His-Asp-Phe-Phe-Val-Gly-Leu-Met-NH_2
thrombin / protease-activated receptors	PAR1, PAR2, PAR3, PAR4	thrombin, trypsin, factor Xa	protein

(Table 1). contd.....

GPCR	code	native ligand (peptide/protein)	nature of the ligand
vasopressin receptors	V_{1A}, V_{1B}, V_2	vasopressin	Cys-Tyr-Phe-Gln-Asn-Cys-Pro-Arg-Gly-NH_2
oxytocin receptor	OT	oxytocin	Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly-NH_2
vasotocin receptor	VT	vasotocin	Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Arg-Gly- NH_2
orexin receptors	OX_1, OX_2	orexin A/B	33 aa/28 aa peptide amides
FSH receptor	FSH receptor	follicle-stimulating hormone (FSH)	protein
LSH receptor	LSH receptor	lutropin, choriogonadotropic hormone, lutenizing hormone	protein
TSH receptor	TSH receptor	thyrotropin, thyroid-stimulating hormone	protein
LHRH receptor	LHRH receptor	gonadotropin-releasing hormone (GnRH), luteinizing hormone-releasing hormone (LHRH)	pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly- NH_2
thyrotropin-releasing hormone & secretagogue receptors	TRH_1, trh_2	thyrotropin-releasing hormone/factor (TRH/F)	pGlu-His-Pro-NH_2
GHS receptor	$GHSR_{1a}$, $GHSR_{1b}$	growth hormone secretagogues (GHS)	oligopeptides
calcitonin/calcitonin gene-related peptide receptors	CGRPR	calcitonin, calcitonin gene-related peptide (CGRP)	32 aa peptide amide
amylin receptor	amylin receptor	amylin	37 aa peptide amide
adrenomedullin receptor	adrenomedullin receptor	adrenomedullin	52 aa peptide amide
corticotropin-releasing factor receptor	CRF_1, CRF_2	corticotropin-releasing factor (CRF)	41 aa peptide amide
gastric inhibitory peptide receptor	gip receptor	gastric inhibitory peptide (GIP)	42 aa peptide
glucagon/glucagon-like peptide receptor	GLP1	glucagon	29 aa peptide
growth hormone-releasing hormone receptor	GHRH receptor	growth hormone-releasing hormone/factor (GHRH/GRF)	44 aa peptide amide
parathyroid hormone receptor	type 1, type 2	parathyroid hormone (PTH)	84 aa peptide
secretin receptor	secretin receptor	secretin	27 aa peptide amide
vasoactive intestinal peptide & PACAP receptor	$VPAC_1$, $VPAC_2$, PAC_1	vasoactive intestinal peptide (VIP) pituitary adenylate cyclase activating peptide (PACAP)	28 aa peptide amide 38 aa peptide

Angiotensin-II Antagonists

Biomedical Significance

The endogenous octapeptide hormone angiotensin-II (A-II) (Table 1), Asp-Arg-Val-Tyr-Ile-His-Pro-Phe, is the key effector compound of the renin-angiotensin system (RAS) which is one of the main blood pressure and electrolyte/fluid homeostasis regulating system in mammals [39]. As a result of a proteolytic cascade starting with angiotensinogen, angiotensin-II is released from its precursor decapeptide angiotensin-I by the action of angiotensin-I converting enzyme (ACE), the latter being a qualified target of antihypertensive drugs [40]. The conversion from angiotensinogen to angiotesin I is catalyzed by the aspartic protease renin, peptide-type inhibitors of which have not yet reached an advanced state of clinical development [41]. A-II interacts specifically with two different receptor subtypes of the GPCR superfamily, notably the AT_1 and the AT_2 receptor, respectively [21]. Interaction with the AT_1 receptor causes severe vasoconstriction, aldosterone release, vasopressin secretion, and renal sodium reabsorption. These effects convergently result in a dramatic increase of extracellular fluid volume, thus giving rise for a significant hypertensive effect. Therapeutic intervention into the RAS clearly offers major clinical and commercial success as shown with the ACE inhibitors for the treatment of hypertension and congestive heart failure [40]. Due to the fact that ACE inhibitors cause dry cough and angioedema [42], new strategies have been sought to block the vasocontrictory activities of the biologically active player, A-II [43]. Specific inhibition of the A-II target receptor interaction, the final step of the RAS, offers an entirely new and selective approach to blocking this regulatory system regardless of the source of the biological active peptide. And indeed, selective nonpeptide A-II antagonists emerged as a new class of

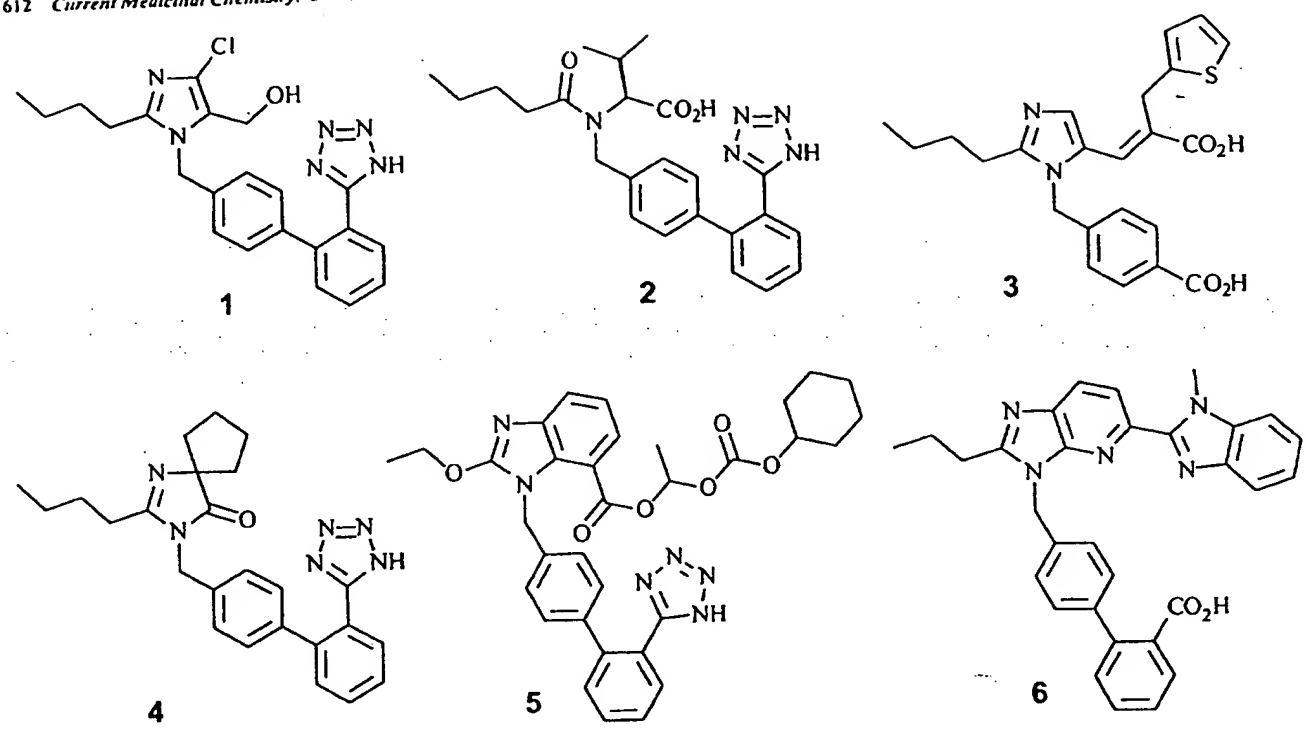


Fig. (5). Structures of marketed AII antagonists.

antihypertensives on the cardiovascular drug market exemplified by the released drugs Losartan **1** [44,45], Valsartan **2** [46], Eprosartan **3** [47], Irbesartan **4** [48], Candesartan **5** [49], and Telmisartan **6** [50], respectively (Fig. (5)).

Consequently, the angiotensin receptor represents one of the most advanced drug targets from the family of peptide-binding (non-opioid) GPCRs in the sense that screening hits have successfully been transferred to leads, further to development candidates that finally reached the drug market as save and innovative drugs introducing a new therapeutic principle.

Lead Finding

In the search for A-II antagonists potent peptides have been synthesized in a classical ligand-based design concept, yielding e.g. [Sar^1,Ala^8]-Angiotensin-II, commonly termed Saralasin [51]. However, all these peptides display limited therapeutic value as potential antihypertensives due to their poor oral bioavailability, rapid excretion, structural complexity, and significant agonistic profiles [51,52].

The feasibility of identifying nonpeptide AT receptor binding compounds with purely antagonistic profile was demonstrated by a research group at Takeda Chemical Industries in 1982. In a series of two patents, Furukawa and co-workers reported on the inhibition of angiotensin-II-induced contractile response in rabbit aorta by numerous different 1-benzylimidazole-5-acetic acid derivatives (Fig. (6)) [53]. The two compounds S-8307 **7** and S-8308 **8** mark the beginning of a new era of antihypertensive drug research in which almost any pharmaceutical company attempted to derive new compounds from that initial findings.

Drug Development

The Takeda compounds served as lead structures for the development of highly potent and selective analogues at DuPont that culminated in Losartan **1** (DuP-753, EXP-7711), the first nonpeptide A-II antagonist that got approval by the FDA and reached the market (Fig. (7)). Guided my molecular modeling studies, the substitution pattern of the benzylic phenyl-ring was changed yielding EXP-6155, **9** which displayed a ten-fold increased binding affinity over e.g. S-8307 **7** [54]. Further extension in *para*-position of the

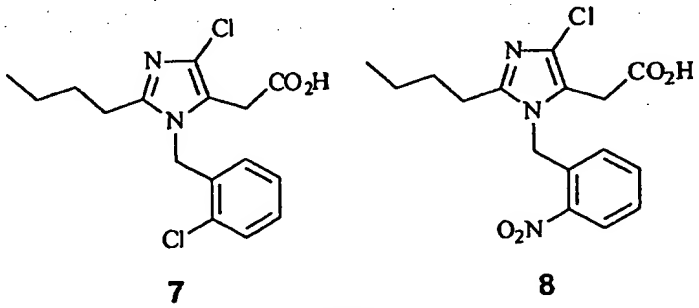


Fig. (6). Initial lead structures disclosed by Takeda Chemical Industries.

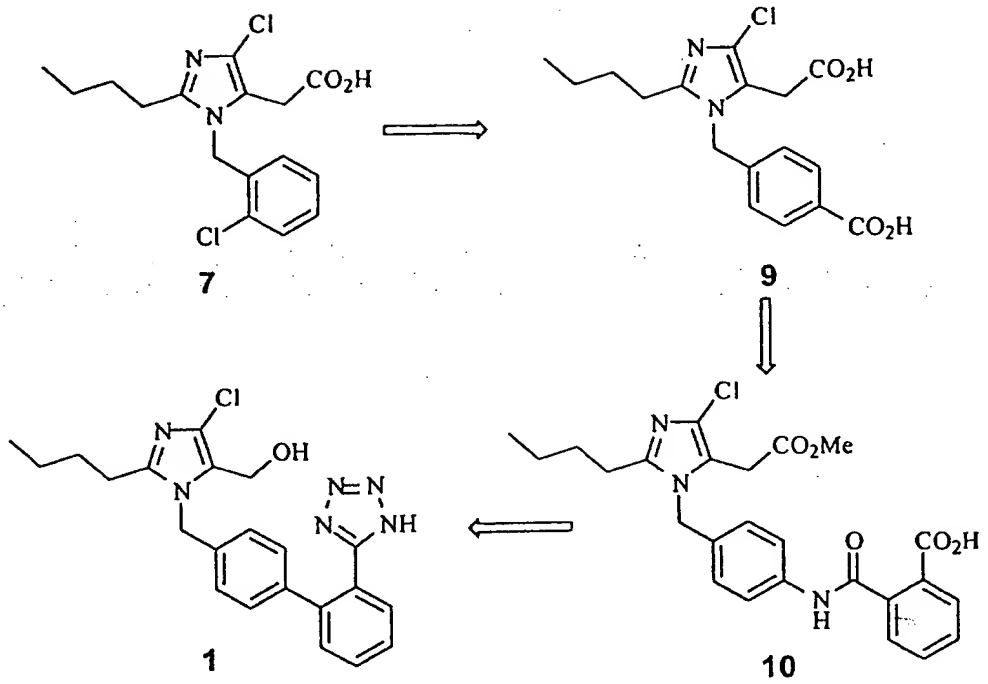


Fig. (7). Development of Losartan 1.

aromatic ring resulted in more potent analogues as shown with EXP-6803 **10** [55].

The deletion of the interaromatic carboxamide linkage yielding biphenylmethyl-substituted imidazole-5-acetic acid derivatives produced orally active compounds and subsequent exchange of the *ortho*-carboxylic acid on the terminal aromatic ring against the tetrazole moiety further improved the oral activity [56,57]. The imidazole-5-acetic acid substituent was modified to the corresponding alcohol in the analogue chosen as clinical candidate. However, later it could be shown that the parent acetic acid sidechain of the imidazole core is the active metabolite of Losartan **1** [58].

Instead of modifying the N-1 substituent of the Takeda imidazole derivatives, **7** and **8**, SmithKline Beecham decided to explore the 5 position in more detail (Fig. (**8**)). Introduction of an acrylic acid in that position (**11**) resulted in a 15-fold enhancement in binding affinity. Further introduction of a 2-thienylmethyl group in α-position of the

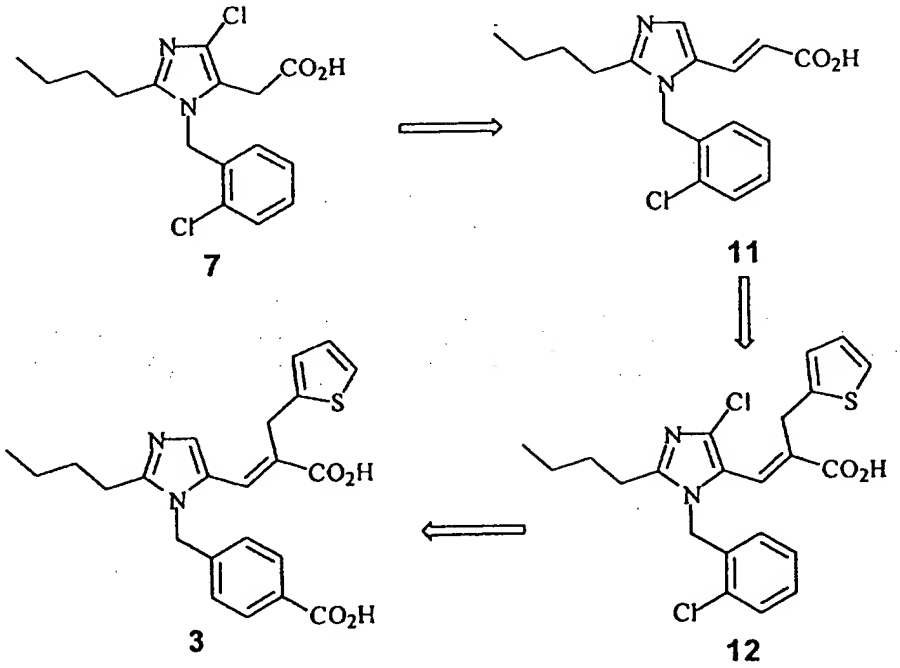


Fig. (8). Development of Eprosartan 3.

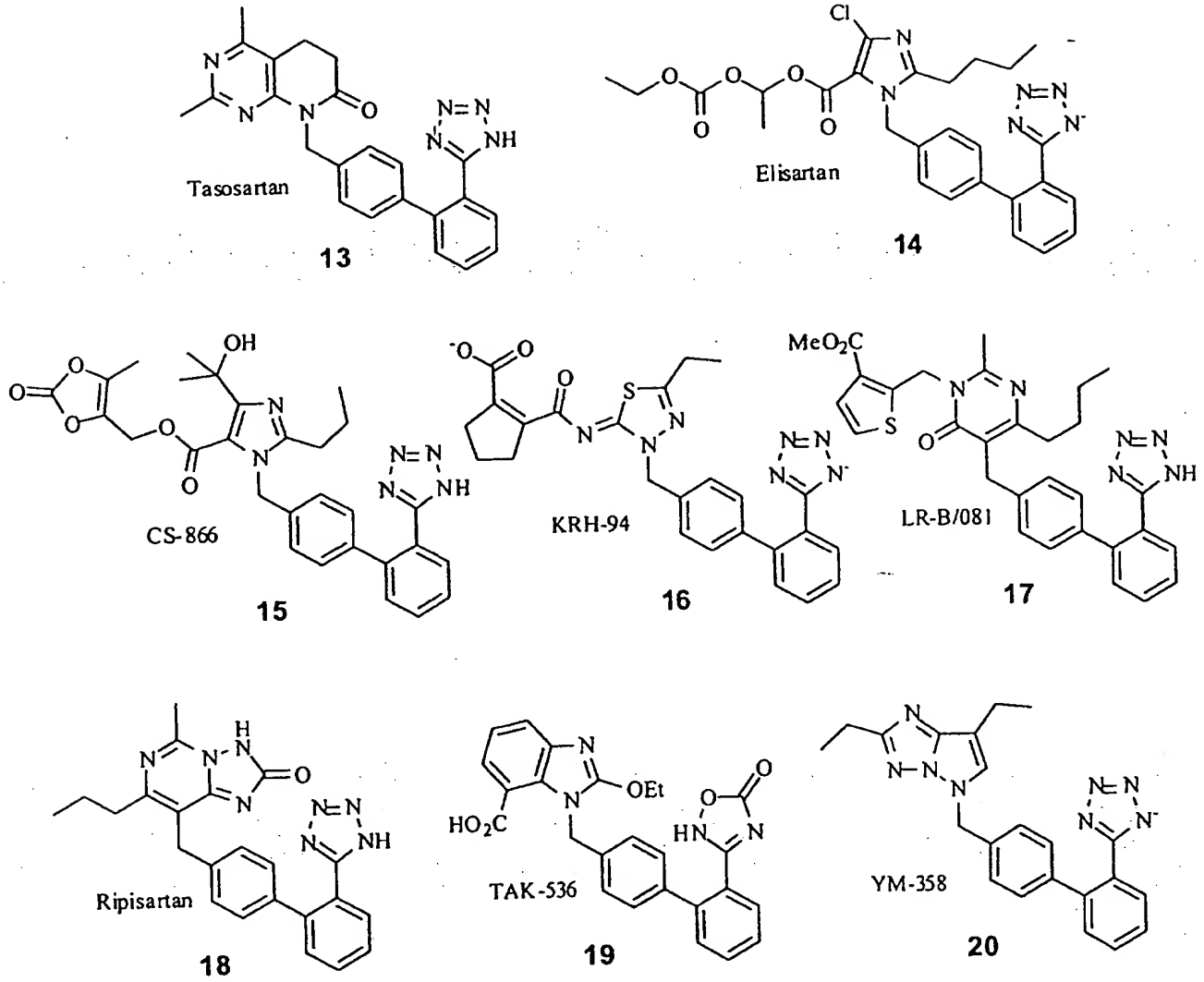


Fig. (9). Next-generation "sartans" in advanced states of clinical development.

acrylic acid substituent (12) together with a modification in the N-1 benzylic substituent finally yielded SK&F-108566, 3 [59,60] which inhibits A-II binding to its receptor in the single digit nanomolar range [61].

The Ciba compound CGP-48933, 2 (Fig. (5)) is the result of an optimization process attempting to replace the imidazole ring structure originally described by Takeda [53]. The 1-benzyl-2-butyl-4-chloro-imidazole-5-acetic acid is replaced with an *N*-terminally acylated amino acid, notably valine. CGP-48933, 2 has passed the clinical development and reached the market as Valsartan [62]. It is clearly beyond the scope of this review to systematically summarize the lead optimization programs pursued by the different pharmaceutical companies, however, it should be emphasized that, apart from the currently marketed drugs, numerous next-generation compounds and follow-ups in late clinical development are expected to get approved in the near future (Fig. (9)) [63,64]. These new "sartans" (13 - 20) together with the first generation drugs (1 - 6) will further change the landscape of antihypertensive prescription drugs since they clearly introduced a new quality of antihypertensive principles into therapy of cardiovascular diseases.

Apart from these biomedical aspects, the development of the "sartans" acting specifically on a member of the GPCR superfamily evolved to a textbook example of protein-targeted drug design within modern medicinal chemistry [65].

Endothelin

Biomedical Significance

Endothelin 1 (ET-1) is a 21 amino acid bicyclic peptide (Table 1) that was initially isolated from porcine aortic endothelial cells [66]. The endothelins constitute a class of three related isopeptides (ET-1, ET-2, ET-3) [67], exhibiting vasoconstrictive and mitogenic potential [68] upon binding to two receptor subtypes, notably the ET_A and ET_B receptor [69,70]. ET-1 selectively binds to the ET_A receptor which is expressed on vascular smooth muscle cells

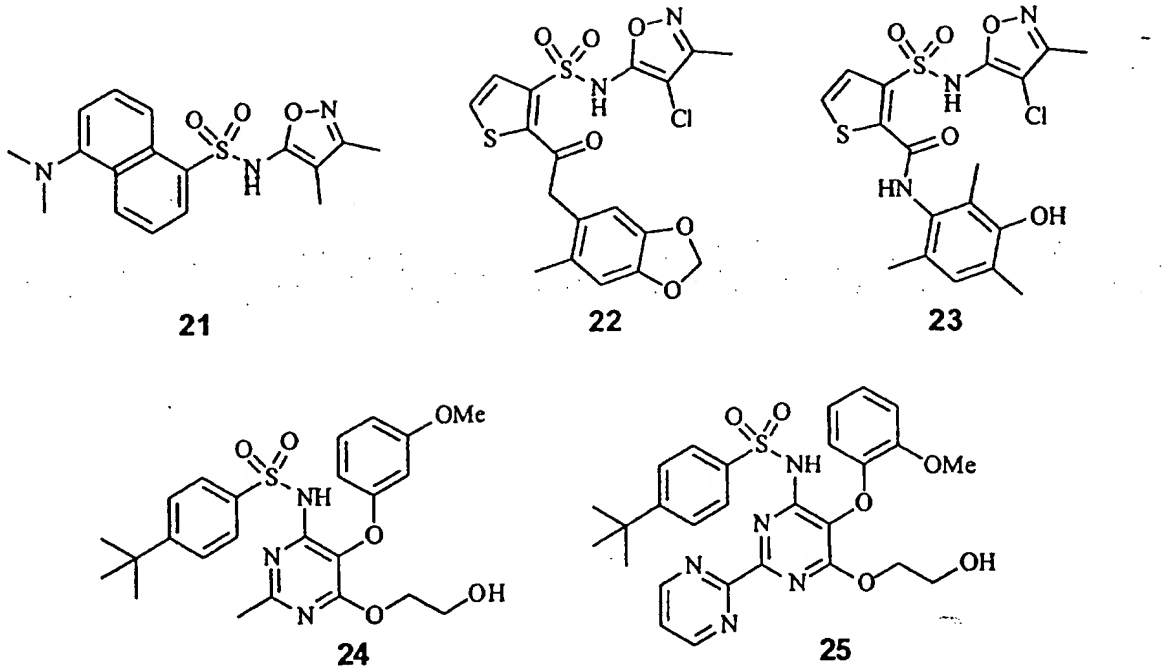


Fig. (10). Aryl-sulfonamide-type ET antagonists.

(lung, aortic, heart) and mediates vasoconstriction and proliferation through activation of a complex intracellular signalling cascade [71,72]. The ET_B receptor, localized in the brain, on vascular endothelial cells, and smooth muscle cells, is responsible for vasodilation via the release of nitric oxide, prostacyclin, and adrenomedullin [73,74]. In addition, ET_B functions as a clearance receptor for endogenous ET by the internalization of the receptor-ligand complex. On the other hand, ET_B may also cause vasoconstriction in some tissues [75]. ET_A and ET_B receptors share high sequence similarity (app. 68%). ET-1 is predominantly produced by endothelial cells acting in an autocrine and paracrine fashion as a mediator of vascular function. Elevated ET levels has been observed in tissue and plasma in a number of cardiovascular disorders, thereby contributing to disease states including hypertension [76], vasospasm, atheriosclerosis [77], acute myocardial infarction [78], congestive heart failure [79,80], restenosis [81], subarachnoid hemorrhage, ischemia, pulmonary hypertension [82], and renal failure [83]. Due to the pivotal pathophysiological role of the endothelin receptor-ligand interaction, this receptor system emerged as a promising target for therapeutic intervention in the disease states mentioned above [84].

Lead Finding

Since the discovery of ET-1 in 1988, a large number of potent antagonists have been described [84]. The first antagonists emerging from random screening efforts have been reported in 1992. These first generation compounds comprise anthraquinones from *Streptomyces misakiensis*, steroids isolated from bayberry, *Myrica cerifera*, and diphenyl ethers discovered in fungal broths [85]. Lead finding in this field is mainly based on compound library screening followed by classical lead optimization within medicinal chemistry programs. A number of peptide-based antagonists have been reported including the prominent cyclic pentapeptide BQ-123, and other peptide antagonists, e.g. BQ-788, FR-139317, PD145065, PD156252, RES-701-1, TAK-044, and IRL2500 [84-88].

As mentioned above, this review, will focus on the development of nonpeptide antagonists emerging from those programs directed towards the discovery of active low molecular weight compounds. Primarily, the ET_A-selective antagonists as well as antagonists exposing mixed ET_A/ET_B affinity play a major role for therapeutic intervention, even though some ET_B-selective antagonists have been reported only recently.

Aryl Sulfonamides

Bristol Myers Squibb designed BMS182874, **21**, a nonpeptide ET_A-selective antagonist from an initial hit which was discovered by screening of a sulfathiazole library [89]. The sulfonamide BMS182874, **21**, exhibits an IC_{50} value of 150 nM at the ET_A receptor (A10 cells) and shows no binding affinity to the ET_B receptor (Fig. (**10**)).

From a similar series of compounds, Immunopharmaceuticals (Texas Biotech.) developed an isoxaolyl-thiophene sulfonamide, TBC-11251, **22** (Sitaxsentan) [90]. This orally active compound has shown efficacy in phase II clinical trial of congestive heart failure (CHF) and demonstrated activity in a rat model of myocardial infarction and acute hypoxia-induced pulmonary hypertension (PH) [91]. Further investigations established a unique pharmacophore framework, characterized by a central thiophene subunit for selective ET_A antagonism [92]. Maintaining the sulfonamide substituent in position 3 and altering the substituent in position 2 in the thiophene ring

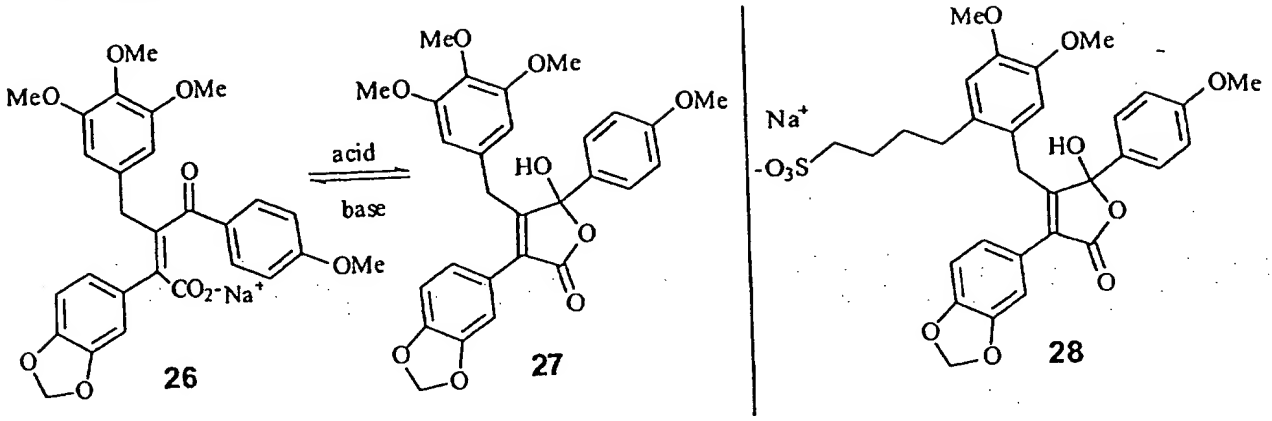


Fig. (11). Butenolide-type ET antagonists.

led to a series of compounds with enhanced pharmacological properties. TBC-2576, **23**, the optimal analogue in this series showed about 10-fold higher ET_A binding affinity compared to Sitaxsentan, **22**, and high ET_A-selectivity, as well as a serum half-life of 7.3 h in rats, paired with *in vivo* activity (Fig. (**10**)) [92].

A number of nonpeptide ET_A/ET_B antagonists based on a pyrimidyl-benzene sulfonamide scaffold have been reported. The first example for an orally active representative is Ro46-2005, **24** [93] which was obtained after optimization of a lead compounds identified by random screening in an antidiabetic project. The binding affinities of Ro46-2005, **24** (K_i=220 nM (ET_A), K_i=1000 nM (ET_B)) could further be optimized yielding the bipyrimidyl-benzene analogue Ro47-0203, **25** (Bosentan) which represents an improvement in both, receptor binding affinities (K_i=4.7 nM (ET_A), K_i=95 nM (ET_B)) and oral activity (Fig. (**10**)) [94]. Bosentan **25** is a competitive mixed ET_A/ET_B antagonist and shows promising results in clinical trials [88] in terms of vasodilation. Further, it improves left ventricular performance and reduces renal dysfunction. The beneficial effects of Bosentan **25** have been characterized in CHF models, in hypertension related experiments and in subarachnoid hemorrhage (SAH) trials. These and other potential applications have been described in a recent review by Roux *et al.* [88].

Butenolides

CI-1020, also known as PD156707, **26, 27** [95] emerged from the optimization of an initial lead structure which was identified from library screening (Fig. (**11**)). The optimization procedure was guided by following the *Topliss* "decision tree" approach based on QSAR principles [96]. CI-1020, **26, 27** represents the first clinical candidate emerging from the Parke-Davis series of butenolides. With an IC_{50} value of 0.30 nM on recombinant human ET_A receptor (IC_{50}=780 nM (ET_B)) it demonstrates high ET_A-selectivity (2600-fold). CI-1020, **26, 27** undergoes tautomerization, thereby establishing the γ-hydroxy butenolide structure **27** under acidic conditions, while at basic pH the equilibrium is shifted in favour of the ring-opened γ-keto acid salt structure **26** [95]. The poor water-solubility of this compound, caused by cyclization, has driven the drug development process towards a series of water-soluble ring-closed γ-hydroxy butenolides applicable for parenteral use [97]. One of the follow-up compounds exhibits promising pharmacological profiles by displaying improved activity compared to CI-1020, **26, 27** e.g. in preventing acute hypoxia-induced pulmonary hypertension (PH) in rats.

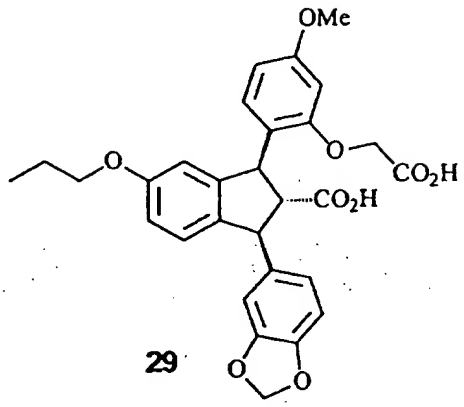
Most promising characteristics were found for an analogue containing the sodium salt of a sulfonic acid in compound **28** (Fig. (**11**)) [97]. It shows high ET_A-selectivity (4200-fold) with an IC_{50} value of 0.38 nM (ET_A) and ET_A functional activity of K_B=7.8, which is similar or even superior to the progenitor CI-1020 **26, 27**. Moreover, it displays improved water-solubility and shows higher activity after *i.v.* infusion in preventing acute hypoxia-induced PH in rats (ED_{50}=0.3 μg/kg/h) when compared to CI-1020 **26, 27** [97]. The new compounds are currently evaluated in preclinical trials, while CI-1020 **26, 27** has already been tested in a model of acute stroke and has entered clinical development for cerebral ischemia.

Indane Carboxylic Acids

SB209670 **29** emerged from the SmithKline Beecham laboratories after optimization of an initial hit discovered from compound library screening (Fig. (**12**)) [98]. Within a molecular modeling-driven approach based on a comparison of the NMR-derived conformation of ET-1 with the primary hit, an indene carboxylic acid derivative, the mixed ET_A/ET_B receptor antagonist SB209670 **29** was designed (K_i=0.43 nM (hET_A), K_i=14.7 nM (hET_B)). When administered *i.v.* SB209670 **29** shows efficacy in different animal models of ET-mediated disease states, e.g. renal failure, hypertension [84], and ischemia-induced stroke. Due to the low oral bioavailability (4%) a structurally related analogue, SB217242 **30** [99] was investigated that displays improved pharamacokinetics and bioavailability [86]. SB209670 **29** is under development (phase I) for acute *i.v.* indications with efficacy in pulmonary hypertension (PH), chronic renal failure (CRF) and stroke [87], while SB217242 **30** (phase I) is in development for chronic PH and chronic obstructive pulmonary disease (COPD) [87,100].

Pyrrolidine Carboxylic Acids

The SmithKline Beecham compound SB209670 **29** (Fig. (**12**)) served as template for the design of the



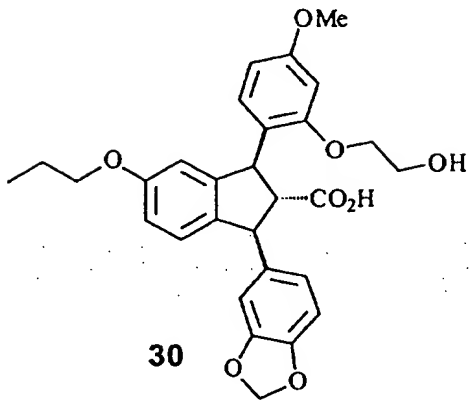


Fig. (12). Indane carboxylic acid-type ET antagonists.

pyrrolidine carboxylic acid A-127722 rac-31 (Fig. (13)) [101], that has been disclosed as a potent, ET_A-selective antagonist, currently tested in clinical trials (PH, CHF) [87]. A-127722 rac-31 was reported to prevent dose-dependently cerebral oedema in stroke-prone spontaneously hypertensive rats [100]. ABT-627 31, the active enantiomer (2*R*,3*R*,4*S*) of the *trans-trans* configurated 2,3,4-trisubstituted pyrrolidine ring, shows an IC_{50} value of 0.08 nM on ET_A and 8.1 nM on ET_B [102]. The 1800-fold selectivity was dramatically altered by subtle structural modifications of A-127722 rac-31, which led to A-182026 32 with an ET_A/ET_B selectivity ratio of 3, thus being the most potent balanced dual ET_A/ET_B antagonist known today. Replacement of the dialkyl-acetamide (rac-31) against a 2,6-dialkyl-acetanilide resulted in an ET_B-selective antagonist, A-192621 33 exhibiting promising pharamcological properties [103]. Combination of the structure-activity relationships (SAR) derived from the first series of ET_A-selective compounds (e.g. ABT-627 31) and the second series of ET_B-selective antagonists (e.g. A-192621 33) led to a further optimized series of compounds. Therein A-308165 34 has been identified as highly selective (27000-fold), orally acitve ET_B antagonist [104].

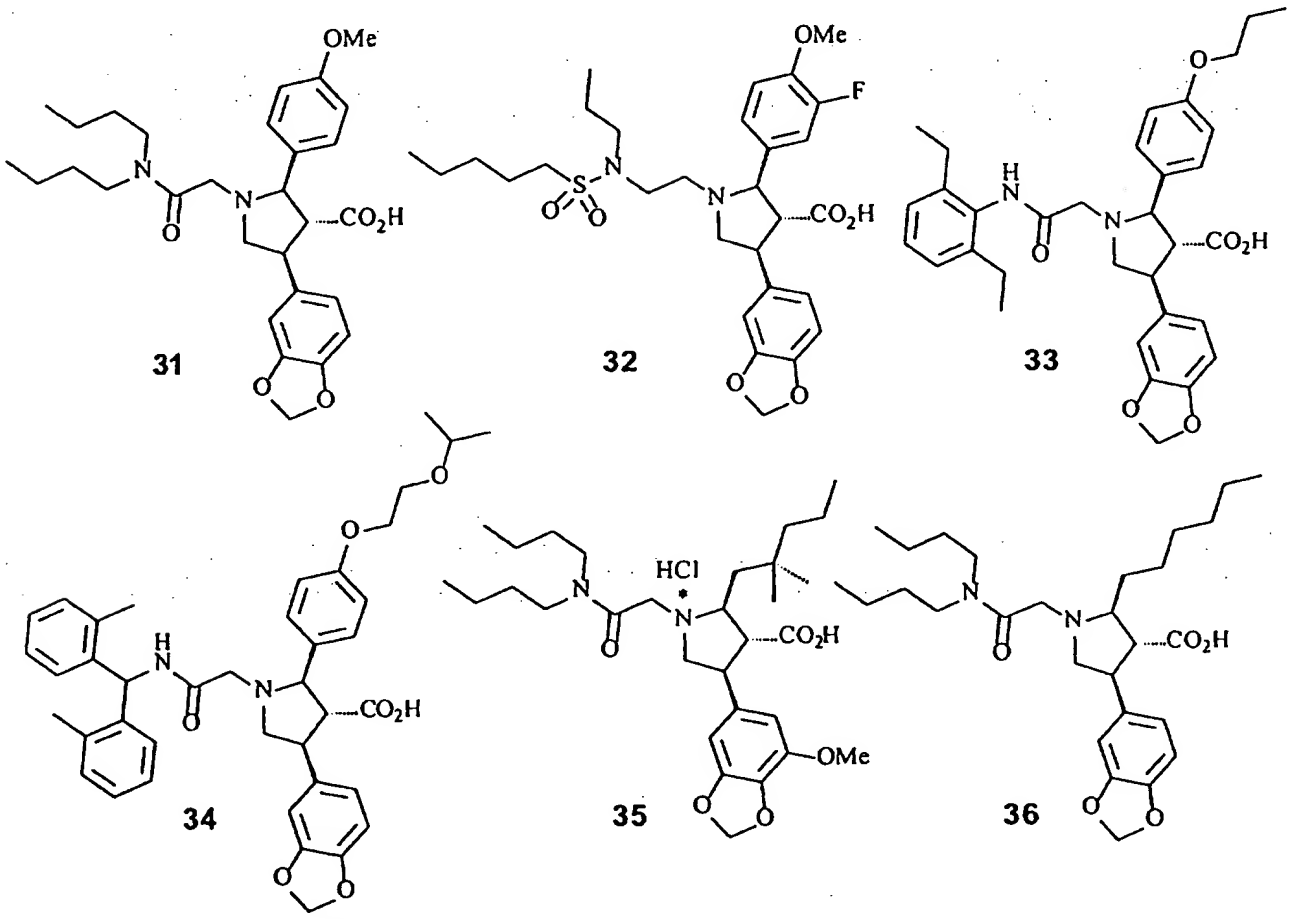
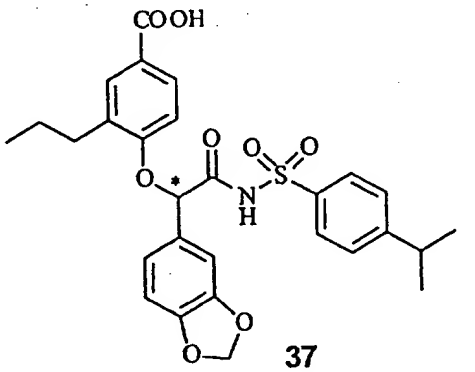


Fig. (13). Pyrrolidine carboxylic acid-type ET antagonists.

Administration of ET_B-selective antagonist led to hypertensive responses indicating that they are not suitable as agents for a long-term systemic single ET_B-directed therapy [103]. Nevertheless, ET_B-selective antagonists are expected to be a valuable tool for the elucidation of the role of the ET_B receptor action under normal and pathophysiological conditions [104]. Most recently, an ET_A-selective antagonist, derived by optimization of A-127722 rac-31, emerged from the series of pyrrolidine-based compounds [105]. A-216546 35 is a further orally active ET receptor antagonist showing >25000-fold selectivity for the ET_A receptor (K_i=0.46 nM), and is considered for clinical development as a therapeutic agent for chronic treatment of ET-1-mediated diseases [106]. Compound 36 (IC_{50}=5.6 nM (ET_A); >10000-fold selectivity) is currently under investigation at Abbott's Laboratories as ET_A antagonist. Apart from the ET receptor affinity, A-216546 35 showed remarkable inhibition potential for numerous members of the GPCR superfamily such as adenosine receptors, δ-opioid receptor, purinergic receptor, etc. [106], thus indicating a kind of "ligand crosstalk" which turns out to be a common phenomenon of GPCR-targeted compounds.

Phenylacetamides

L-749,329 37 (Fig. (14)) is an orally active, competitive and nonselective ET_A/ET_B antagonist developed by Merck inhibiting the binding of [^{125}I]ET-1 in Chinese Hamster Ovary (CHO) cells expressing human ET receptors with IC_{50} values of 0.8 nM (ET_A) and 16 nM (ET_B), respectively [107]. The active enantiomer, L-754,142 37, is a potent orally active ET antagonist with a long duration of action in several *in vivo* models. L-754,142 37 shows binding affinity



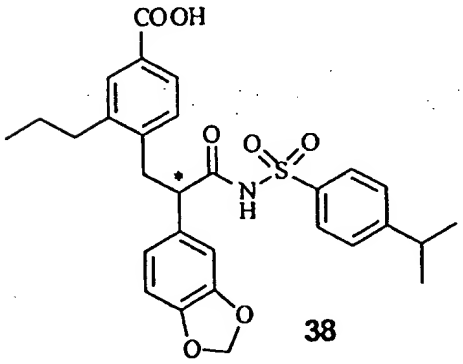
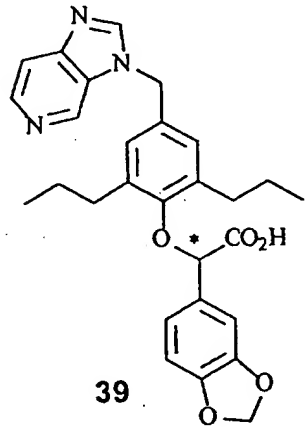


Fig. (14). Phenylacetamide-type ET antagonists.

towards ET_A (0.062 nM) and ET_B (2.25 nM) and antagonizes ET-1-induced phosphatidyl inositol hydrolysis in CHO cells expressing cloned human ET receptors with IC_{50} values of 0.35 nM (ET_A) and 26 nM (ET_B) [108]. Substitution of the ether oxygen against a methylene group resulted in L-751,281 38, an analogue with similar activities on both ET receptor subtypes [107].

α-Phenoxyphenylacetic Acids

At the Merck laboratories, structural modifications of an initial lead discovered by screening for angiotensin II (AII) antagonists, led to a dual AT_1/ET antagonist. Further optimization towards ET_A-selectivity resulted in L-744,453 39 (Fig. (15)), an α-phenoxyphenylacetic acid derivative lacking the sulfonamide present in the arylacylsulfonamides L-749,329 37, and L-751-281 38 [107]. L-744,453 39 competitively and reversibly inhibits [^{125}I]ET-1 binding to CHO cells expressing cloned human ET receptors with K_i values of 4.3 nM (ET_A), and 232 nM (ET_B). Thus, within L-744,453 39 the shift from an originally angiotensin II antagonist to an ET-selective antagonist could be demonstrated, thus highlighting the potential of "cross-fertilization" of projects devoted to representatives of a common receptor superfamily.



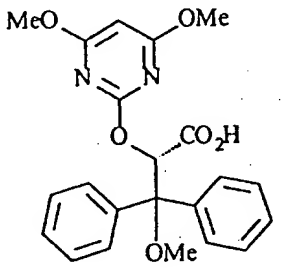


Fig. (15). Aryloxyacetic acid-type ET antagonists.

α-Aryloxyacetic Acids

Also at the BASF laboratories, the endothelin project started with screening of the in-house chemical substance stock. The initial lead, which was originally intended as a herbizide, was optimized by systematic structural modifications resulting in an ET_A-selective antagonist, LU135252 40 (Fig. (15)), the active (*S*)-configurated enantiomer of LU127043 [109,110]. It selectively binds to the ET_A receptor with high affinity (K_i=2 nM (ET_A), K_i=184 nM (ET_B)) [111]. LU135252 40 has been evaluated in clinical trials for preventing restenosis [87] and entered phase II for CHF [112]. Furthermore, it was demonstrated that selective ET_A receptor inhibition with LU135252 40 could reduce ischemia-induced ventricular arrhythmias in pigs. Thus ET antagonism might reduce mortality by preventing arrhythmias, a major cause of death in CHF, obviously induced by the pro-arrhythmogenic effects of ET-1 [100].

Phenoxybutanoic Acids and Stilbene acids

According to a previously elaborated SAR study, Astles *et al.* at Rhône-Poulenc Rorer presented the optimized analogue RPR-111844 **41** (Fig. (**16**)), which exhibits an IC_{50} of 5.0 nM at the rat ET_A receptor and 1000-fold selectivity over the ET_B receptor. The promising pharmacokinetics in a rat model of ET-1-induced vasoconstriction rendered this RPR-111844 **41** an ideal candidate to examine these effects in preclinical models of cardiovascular disease [113].

In order to shed light on the characteristics of the bioactive conformation, a new series of rigidified analogues of stilbene acids were designed based on the SAR derived from a series of the phenoxybutanoic acids. Thus, compound RPR-111723 **42** was identified as the most potent analogue with an IC_{50} of 80 nM. Although the stilbene series was not further developed, results from SAR will be back-transferred into the more interesting series of phenoxy butanoic acids [114].

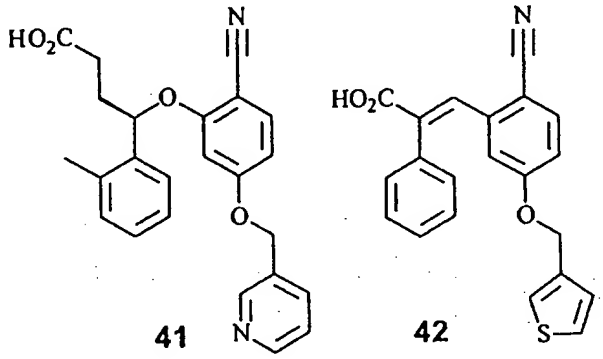


Fig. (16). Phenoxybutanoic acid- and stilbene acid-type ET antagonists.

Bradykinin

Biomedical Significance

The nonapeptide bradykinin (BK, Table 1), Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg, belongs to the family of kinins. Kinins are small peptides which are released from kinninogens by several enzymes, the kallikreins [115-120]. Interaction of BK with two designated receptor subtypes, B_1 and B_2, results in a variety of biological effects including vasodilation, modulation of vascular permeability, smooth muscle contraction, recruitment and priming of inflammatory cells, induction of pain, modulation of transmitter release, stimulation of cell division, etc. [121]. Based on these diverse biological activities, BK is involved in inflammatory diseases, such as asthma, rhinitis, pancreatitis, sepsis, rheumatoid arthritis, brain oedema, and angioneurotic oedema [122]. Due to these pathophysiological actions of BK, mainly induced by the interaction with the B_2 receptor, this system emerged as an interesting target in pharmaceutical research. Hence, in a number of efforts BK antagonists were presented tempted to be a valuable tool in the treatment of above mentioned chronic diseases.

Lead Finding

'Second-Generation' B_2 Antagonists

Initiated by the discovery of NPC-567 by Vavrek and Stewart [123] in the 90's, a number of selective peptidic B_2 receptor anatgonists including Icatibant (Hoe-140) [124,125] and Bradycor (Deltibant, CP-0127) [126], so-called '*second-generation*' antagonists, have been clinically evaluated. In the following years, research programs were directed towards the discovery of B_2-selective nonpeptide antagonists. Detailed overviews on this subject were provided only recently by Altamura *et al.* [127] and Heitsch [128] addressing projects of diverse research group, and reviewing the current patent situation.

In 1993, the naphthylalanine derivative WIN-64338 **43** (Fig. (**17**)) was disclosed as the first nonpeptide B_2 antagonist [129,130]. A random screening approach at Sterling Winthrop led after optimization to compound WIN-64338 **43**, displaying a K_i value of 64 nM for the inhibition of [^{3}H]BK binding to the B_2 receptor (IMR-90 cells, fetal lung fibroblast cell line expressing the kinin B_2 receptor). However, this compound is problematic in terms of potency, oral bioavailability, and selectivity [130], since significant affinity for e.g. the muscarinic receptors was detected [131].

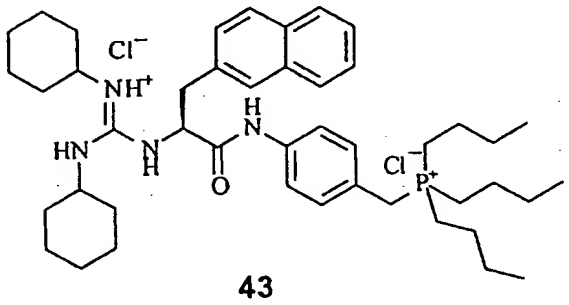


Fig. (17). 'Second-generation' B_2 antagonists WIN64338.

'Third-Generation' B_2 Antagonists

From 1994 on Fujisawa published a series of patent applications on new classes of potent, selective and orally active nonpeptide B_2 receptor antagonists [132-135], thereby establishing the so-called '*third-generation*' compounds. Several derivatives showed nanomolar affinity in receptor binding assays and high efficacy in various species including humans. They also exhibited *in vivo* functional antagonistic activity against BK-induced bronchoconstriction in guinea pigs and potency in diverse animal models of inflammation [132-135] [136,137]. Again, these compounds originally emerged from a random screening directed towards the angiotensin II (AII) AT_1 receptor and belong to a class of imidazo[1,2-*a*]pyridines. A detailed description of the design, synthesis and biological evaluation was given by Kayakiri *et al.*, only recently [138]. The first lead compound **44** (Fig. (**18**)) of this series of *N*-containing heteroaromatic benzyl ethers showed an IC_{50} value of 7.6 μM.

Within a classical medicinal chemistry approach based on SAR considerations the first lead compound **44** was exposed to extensive modifications leading to **45** (Fig. (**18**)).

This analogue displays an IC_{50} value of 2.4 nM for the inhibition of the specific binding of [^{3}H]BK to B_2 receptors in guinea pig ileum (GPI) membrane preparations. Thus, the 8-[3-(*N*-acylglycyl-*N*-methylamino)-2,6-dichlorobenzyloxy-3-halo-2-methylimidazo[1,2-*a*]pyridine skeleton was identified as the basic framework of the first orally active nonpeptide B_2 antagonist. In order to overcome species difference, further modifications within the 3-position of the benzyl moiety revealed an analogue (FR167344 **46**) exhibiting subnanomolar (IC_{50}=0.66 nM) and low nanomolar binding affinities (IC_{50}=1.4 nM) for GPI membrane and human A431 cells (epidermoid carcinoma cells) [136,139], respectively.

Recent results indicate that FR167344 **46** has specific antagonistic activity against guinea pig tracheal smooth muscle BK receptors, thus rendering it a potential therapeutic tool for the treatment of asthma [140]. Derivatives containing the *N*,*N*-dimethylcarbamoyl-substituted cinnamide group were capable of overcoming species differences, and therefore defined the required pharmacophore for further investigations. FR167344 **46** was assigned as new lead compound for three independent optimization approaches implying substitutions within the imidazo[1,2-*a*]pyridine moiety (benzimidazoles, quinoxalines, and quinolines). While further optimization of the quinoxaline series failed, optiization within the benzimidazole and quinoline series resulted in several potent congeners. Thus, consequent SAR studies of the benzimidazoles afforded improvements of *in vivo* oral activities, resulting in FR185627 **47** which exhibits 75.2 % inhibition against BK-induced bronchoconstriction at 0.32 mg/kg, *i.p.* [138]. Optimization of the quinoline series afforded compound FR173657 **48** with high potency in B_2 binding affinities for both GPI (IC_{50}=0.46 nM) and human recombinant B_2 receptors (IC_{50}=1.4 nM) [136,141]. FR173657 **48** displays the best *in vivo* B_2 antagonistic oral activity among nonpeptide antagonists investigated so far

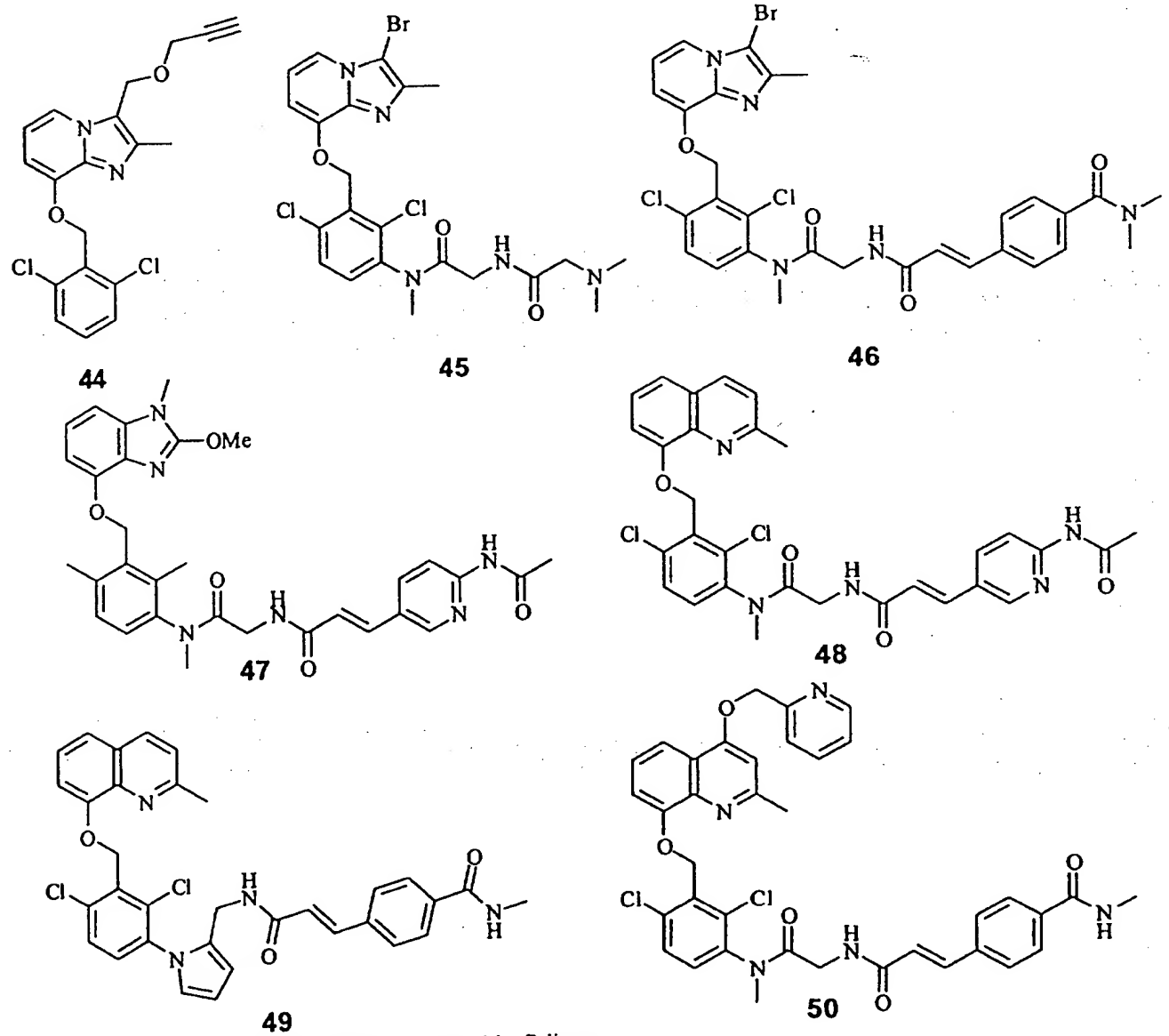


Fig. (18). 'Third-generation' B_2 antagonists developed by Fujisawa.

and was chosen as a clinical candidate for the treatment f various inflammatory diseases. Recent investigations on plasma extravasation mediated by activation of sensory nerves in guinea pig airways suggest FR173657 **48** to be an orally active, promising anti-inflammatory agent for kinin-dependent inflammation following antigen challenge [142]. Fujisawa researchers further report on the postulation of the active conformation of their compounds by synthezising conformationally restrained analogues. Molecular modelling studies and subsequent chemical synthesis of a novel pyrrole series afforded FR193144 **49**, an analogue which mimics the previously postulated *cis*-conformation of the *N*-methylamide by the pyrrole moiety. FR193144 **49** exhibits excellent binding affinity for human recombinant B_2 receptors (IC_{50}=0.26 nM), thereby proving the *cis*-conformation as the bioactive conformation of the *N*-methylamide bearing antagonists (Fig. (**18**)) [138].

Interestingly, only minor variations within the core structure of the B_2 antagonists resulted in an analogue, FR190997 **50** (Fig. (**18**)), exhibiting an agonistic profile [143]. The agonistic behaviour is hypothesized to be encoded in the difference concerning the 4-substituent of the quinoline moiety within the agonist compared to the antagonists (H ⇔ 2-pyridylmethoxy). FR190997 **50** induces hypotensive response in anaesthetized rats and thus, is claimed for the treatment of hypertension, renal failure, heart failure, circulatory disorders, angina, restenosis, hepatitis etc [143].

B_2 Antagonists Structurally Related to FR173657

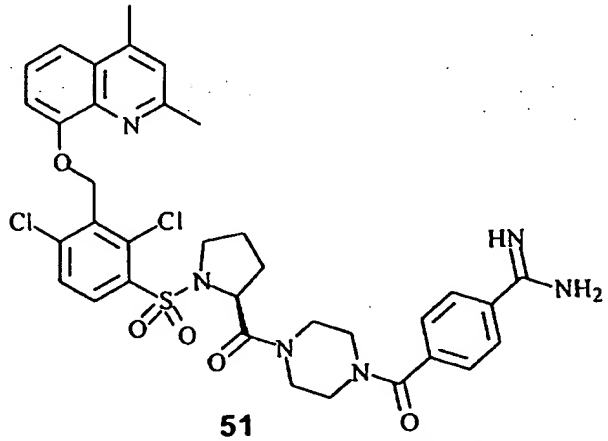
Compounds evaluated at Fournier are structurally related to Fujisawa's quinoline series differing mainly in the substituent in 3-position of the benzene-linkage which is replaced by a sulfonamide. LF16-0335 **51** (Fig. (**19**)) is a potent, selective and competitive antagonist of the human B_2 receptor, displacing [^{3}H]BK binding to membrane preparations of CHO cells expressing cloned human B_2 receptors with a K_i value of 0.84 nM.

LF16-0335 **51** shows neither affinity for the B_1 receptor, nor binds significantly to any other membrane receptor except the muscarinic M2 (IC_{50}=0.9 μM) and M1 (IC_{50}=1.0 μM) receptors [144]. The hydrochloride of this derivative, LF16-0335C, inhibits competitively BK-induced contractions of isolated rat uterus and GPI in functional assays [145]. Given *i.v.*, LF16-0335C inhibits BK-induced hypotension in both animal species in a dose-dependent manner [145]. Substitution of the piperazine ring in LF16-0335 **51** against a diaminopropane unit led to LF16-0687 **52** (Fig. (**19**)) which was shown in competition binding studies with [^{3}H]BK to bind to the human recombinant B_2 receptor expressed on CHO cells with an K_i value of 0.67 nM (LF16-0335 **51**, K_i=0.84 nM). It functions as a competitive antagonist of BK-mediated contractions in isolated organs, i.e. rat uterus and GPI. Contrary to LF16-0335 **51**, LF16-0687 **52** showed selectivity for the B_2 receptor in binding and functional studies performed on more than 40 different receptors.

In a new series of patent applications, Hoechst claimed a number of derivatives based on the lead structures delineated by Fujisawa as potent B_2 receptor antagonists. These heteroarylbenzyl ethers belong to a series of *O*-substituted 8-quinolines or 4-benzothiazoles [146]. Heitsch *et al.* report that the potency of the quinoline series was found to be higher compared to the corresponding benzothiazoles. The most potent antagonist **53** (Fig. (**20**)) shows an IC_{50} value of 0.7 nM for the inhibition of specific binding of [^{3}H]BK to GPI membrane preparations and an EC_{50} value of 4.1 nM for the inhibition of BK-induced contraction of isolated GPI.

The most potent corresponding antagonist of the benzothiazole series **54** (Fig. (**20**)) exhibits an IC_{50} value of 10.3 nM and an EC_{50} value of 54 nM. Another representative example of the B_2 antagonist claimed by Hoechst is compound **55** (Fig. (**20**)) which incorporates a 2-aminoethanol unit instead of the *N*-methylamide as linker in the central part of the molecule. **55** inhibits [^{3}H]BK binding (GPI) with a K_i value of 20 nM [127,128].

Based on the template FR173657 **48**, Kyowa Hakko filed a patent application claiming heteroarylbenzyl ethers as B_2 antagonists [147]. Like in FR173657 **48**, the central ether entity is flanked by a terminal quinoline and a dichlorobenzene linker. Instead of the classical *N*-methylamide sidechain in 3 position, the dichlorobenzene linker bears a branched hydrocarbon chain (**56**, Fig. (**20**)).



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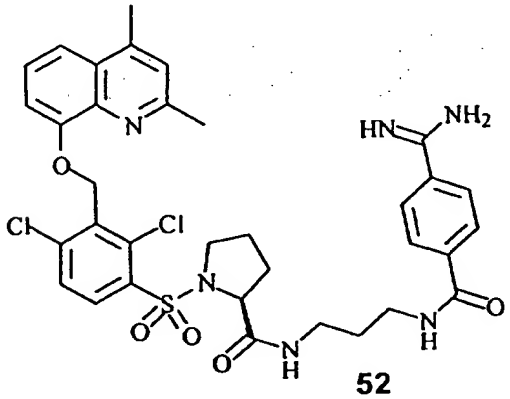

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Fig. (19). B_2 receptor antagonists disclosed by Fournier.

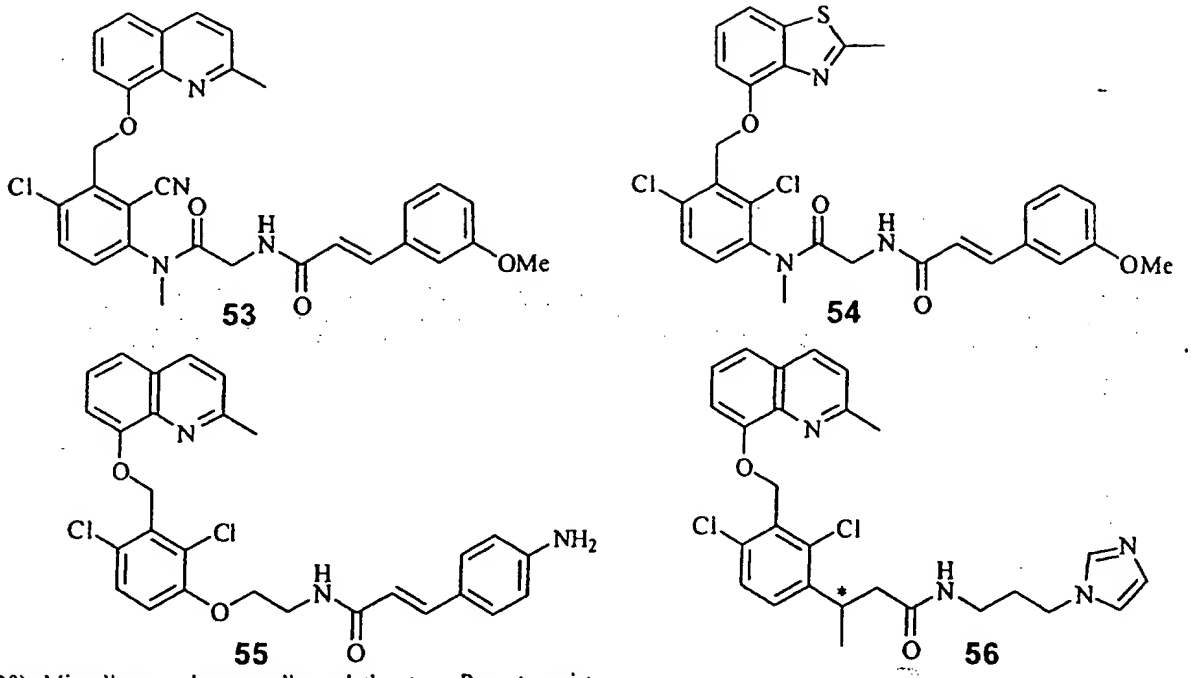


Fig. (20). Miscellaneous heteroarylbenzylether-type B_2 antagonists.

Miscellaneous Nonpeptide B_2 Antagonists

From screening of a 4000 compound combinatorial library, GlaxoWellcome found a promising tetrahydroisoquinoline, GR213548X **57** (Fig. (**21**)), with affinity for the B_2 receptor in the micromolar range [127].

Further B_2 antagonists are claimed in a series of patent applications by a number of companies. American Home Products (AHP, Wyeth Ayerst) presented compound **58** which structurally resembles the Fujisawa derivatives only with respect to a quinoline entity. Pfizer described 1,4-dihydropyridines such as **59** to act as B_2 antagonists, while Eli Lilly disclosed benzothiophenes **60** (Fig. (**21**)) [127,148-150].

Neurokinin

Biomedical Significance

Neurokinins (NKs), also termed tachykinins belong to a family of peptides sharing a common homologous *C*-terminal fragment composed of the pentapeptide amide Phe-

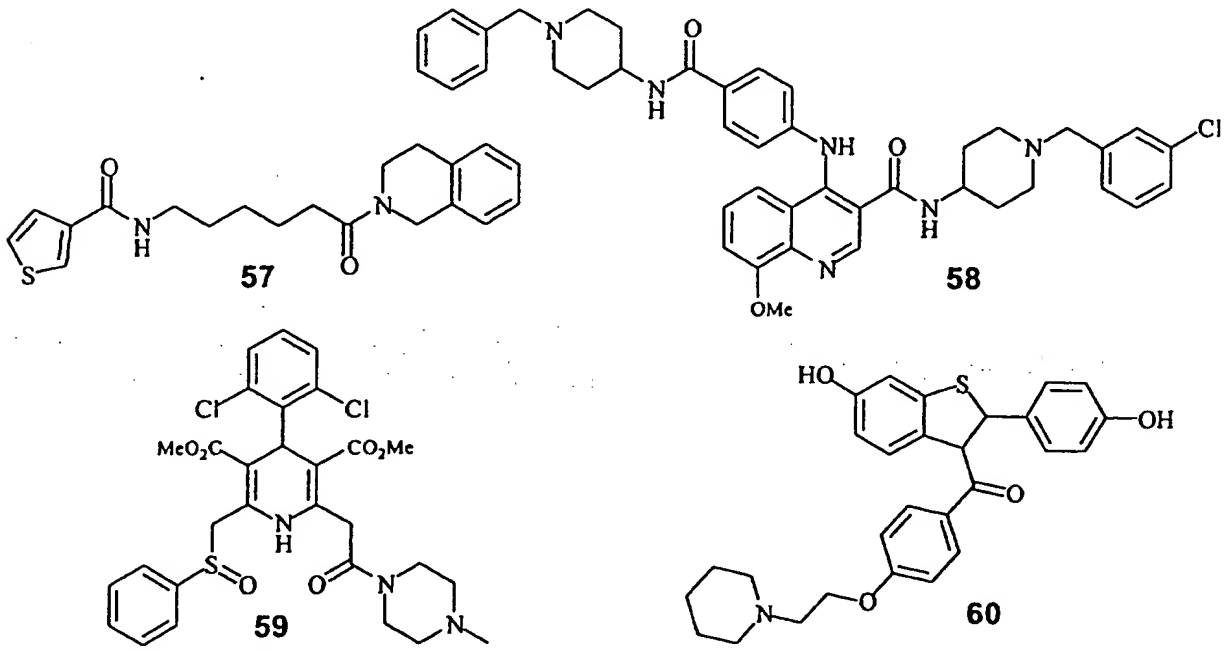


Fig. (21). Miscellaneous B_2 antagonists.

Xaa-Gly-Leu-Met-NH_2 (Table 1) [151]. The interaction of substance P (SP), neurokinin A (NKA) , and neurokinin B (NKB) with their corresponding receptors [152], notably NK_1, NK_2, and NK_3 plays a pivotal role in induction and progression of inflammatory diseases. Neurokinin interaction is involved in a variety of physiological and pathophysiological conditions such as pain, inflammation, smooth muscle contraction, vasodilation, and activation of the immune system. Thus, NK receptor antagonists emerged as interesting agents for the treatment of primarily pain, emesis and asthma but also to interfere in other disorders such as anxiety, arthritis, migraine, cancer and schizophrenia [153-156]. NK receptor antagonists have been reviewed e.g. by Elliot and Seward [157], von Sprecher *et al.* [158], and, only recently, in *Current Medicinal Chemistry* by Gao and Peet [159]. Therefore, this contribution will solely focus on nonpeptide NK antagonists.

Lead Finding

NK_1 Antagonists

The quinuclidine-based analogue CP-96,345 **61** (Fig. (**22**)) was developed from a lead structure found by random screening and is the first nonpeptide NK_1-selective antagonist showing an IC_{50} value of 0.77 nM (lymphoblast IM-9 cells) [160]. Over the last years, CP-96,345 **61** evolved as the main pharmacological tool in the area of NK receptor research.

A second series of piperidine-containing analogues developed at Pfizer includes CP-99,994 **62** [161] and CP-122,721 **63** (Fig. (**22**)) [162]. CP-99,994 **62** exhibits analgesic efficacy [163] and shows less *in vivo* inhibition of NK_1 receptor-mediated responses compared to the 5-trifluoromethoxy analogue, CP-122,721 **63** [164]. The latter

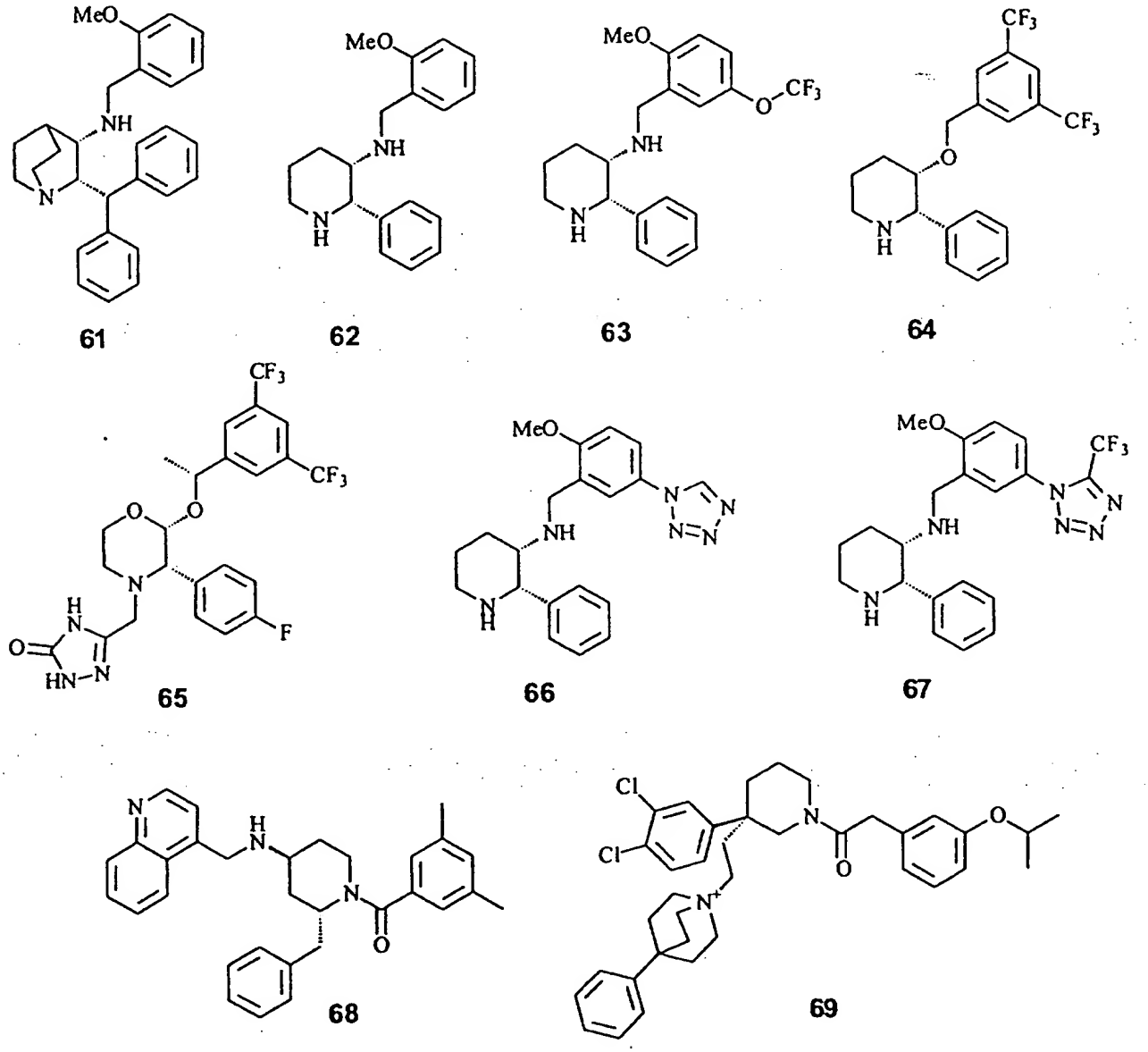
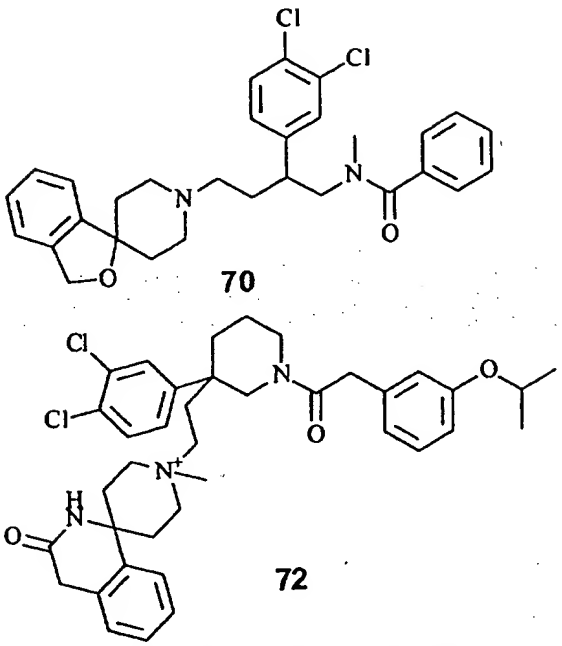


Fig. (22). Quinuclidine-, piperidine-, and morpholine-derived NK_1 antagonists.



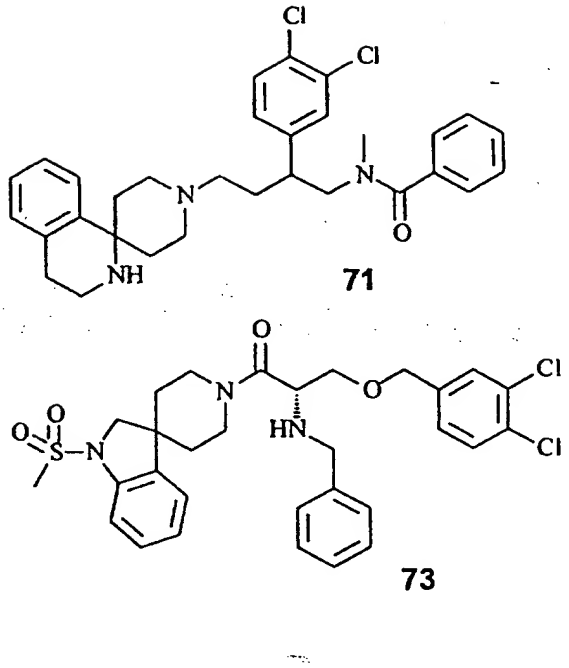


Fig. (23). Spiro-aryl piperidine-type NK_1 antagonists.

congener shows improved antiemetic properties in acute cisplatin-induced vomiting in tumor patients when administered in combination with a 5-HT_3 antagonist [157].

Based on the piperidine core structure of CP-99,994 **62**, Merck synthesized L-733,060 **64** (IC_{50}=0.87 nM in CHO cells) [165] which, after modifications, led to the metabolically more stable L-754,030 **65** (IC_{50}=0.1 nM in CHO cells) (Fig. (22)) [166]. Recent results indicate that L-754,030 **65** prevents cisplatin-induced emesis in patients receiving an anticancer chemotherapy [167,168].

Glaxo disclosed the 5-tetrazolyl-substituted analogue GR-203,040 **66** (Fig. (22)) retaining the piperidine core structure of CP-99,994 **62** as NK_1 antagonist (GR-203,040 **66**: pK_i=10.3 nM in CHO cells) which was selected for clinical evaluation in emesis and migraine [169,170]. Further modification revealed GR-205,171 **67** (Fig. (22)) (pK_i=10.6 nM in CHO cells) which, apart from oral bioavailability, exhibits also reduced L-type calcium channel activity, a side effect associated with e.g. CP-122,721 **63**. GR-203,040 **66** ameliorates tissue damage induced by x-irradiation or cisplatin [171,172].

Novartis developed CPG-49,823 **68** (Fig. (22)), based on the piperidine scaffold for anxiety-related indications [173]. CPG-49,823 **68** (IC_{50}=12 nM, bovine retina) has been tested for its antagonistic potential against the depolarization of spinal motoneurones by bath application of the selective tachykinin receptor against septide(6-11) exhibiting an IC_{50} value of 0.3 μM (gerbil preparations) and 7.8 μM (rat preparations) [174].

The central piperidine unit is also found in the Sanofi compound SR-140,333 **69** (Fig. (22)) (IC_{50}=0.01 nM in IM-9 cells), also termed Nolpitantium, which emerged from a random screening approach followed by a lead optimization program [175].

Investigations on the effects of SR-140,333 **69** on nociceptive pathways in rats revealed this agent to be a potent drug for pain relief [176]. Kubota *et al.* reported on the synthesis of spiro-piperidines as NK_1 receptor antagonists [177]. SAR studies starting from the primary lead YM-35375 **70** (dual NK_1/NK_2 antagonist) (Fig. (23)) yielded analogue YM-35384 **71** as a selective NK_1 antagonist which was 12-fold more potent compared to the original spiro-isobenzofuran-1(3*H*)-4'-piperidine YM-35375 **70**. YM-35384 **71** already showed an IC_{50} value of 58 nM which could be improved by further modification resulting in compound YM-49244 **72** (Fig. (23)), a spiro-substituted piperidinium salt with an IC_{50} value of 1.9 nM against SP-induced contraction in guinea pig ileum and inhibitory activity against selective NK_1 receptor agonist-induced bronchoconstriction in guinea pigs (ID_{50}=24 μg/kg, *i.v.*) [177].

A further class of spiro-aryl piperidines is represented by Merck Sharp and Dohme's spirocyclic aryl sulfonamides, serine-derived NK_1 antagonists [178]. Compound **73** (Fig. (23)) exhibits an IC_{50} value of 1.0 nM for the displacement of [^{125}I]SP from NK_1 receptors in CHO cells and served for

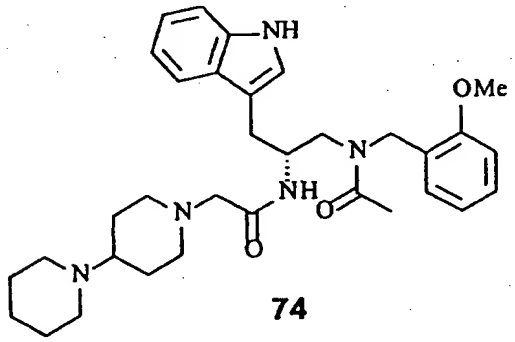


Fig. (24). Lanepitant disclosed by Eli Lilly.

the development of a pharmacophore model for the receptor binding requirements [179].

Eli Lilly has identified the tryptophane-derived LY-303,870 74 (Fig. (24)) as a selective antagonist binding to NK_1 with high affinity, while lacking ion channel activity [180]. LY-303,870, Lanepitant 74, is a candidate for clinical development in animal models of inflammation, pain, migraine, and asthma [158].

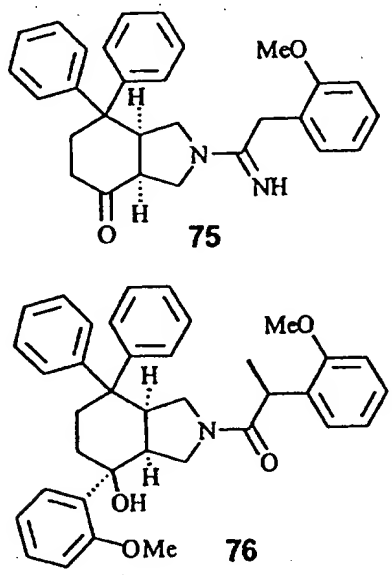


Fig. (25). Perhydroisoindole-type NK_1 antagonists.

RP-67,580 75 (Fig. (25)) emerged after lead optimization of an initial screening hit of Rhône-Poulenc Rorer's compound stock. RP-65,580 75 belongs to a class of substituted perhydroisoindoles which, apart from poor oral bioavailability, also suffered from L-type calcium channel interaction [151,181]. The follow-up compound RPR-100,893 76 (Fig. (25)), Dapitant, exhibits superior binding affinity (IC_{50}=13 nM, IM-9 cells) [182].

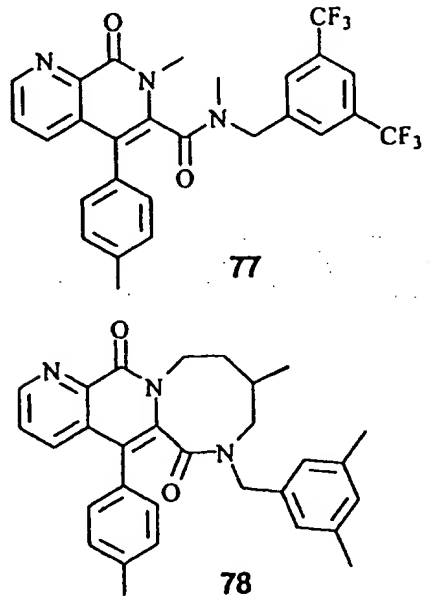


Fig. (26). Naphthydrine-type NK_1 antagonists.

Investigations of the axially chiral 1,7-naphthydrine-6-carboxamide 77 (Fig. (26)) revealed that the atropisomer (*aR*)-trans-77 represents the bioactive receptor-bound conformation of this potent NK_1 antagonist [183]. This analogue exhibits *in vitro* antagonistic activities for the inhibition of [^{125}I]Bolton-Hunter(BH)-SP binding in human lymphoblast cells (IM-9) with an IC_{50} value of 0.24 nM. Further, it shows *in vivo* potency by inhibiting capsaicin-induced plasma extravasation in the trachea of guinea pigs upon *i.v.* and *p.o.* administration.

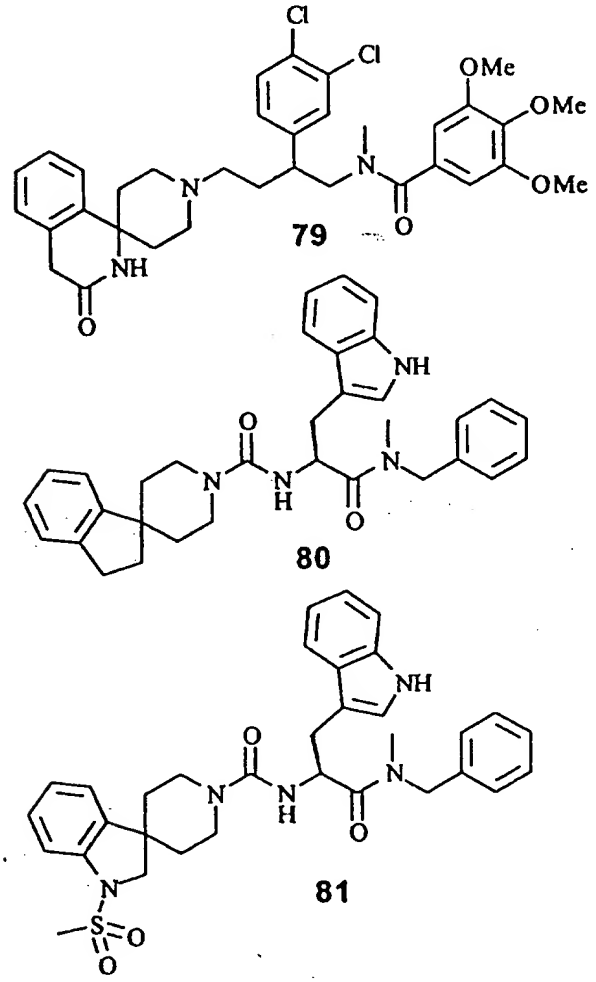


Fig. (27). Dual NK_1/NK_2 antagonists.

Based on this template, Natsugari *et al.* [183] developed TAK-637 78 (Fig. (26)), the (*aR*,9*R*)- atropisomer of a cyclic naphthyridine analogue. TAK-637 78 exhibits an IC_{50} value of 0.45 nM, an ID_{50} of 4.3 μg/kg and 33 μg/kg after *i.v.* and *p.o.* administration, respectively. Further it increased the shutdown time of distension-induced bladder contractions and the bladder volume threshold in guinea pigs, thus implying its clinical potential in the treatment of pollakiuria and urinary incontinence [183]. The x-ray structures of 77 and 78 provide insights in the prerequisite structural

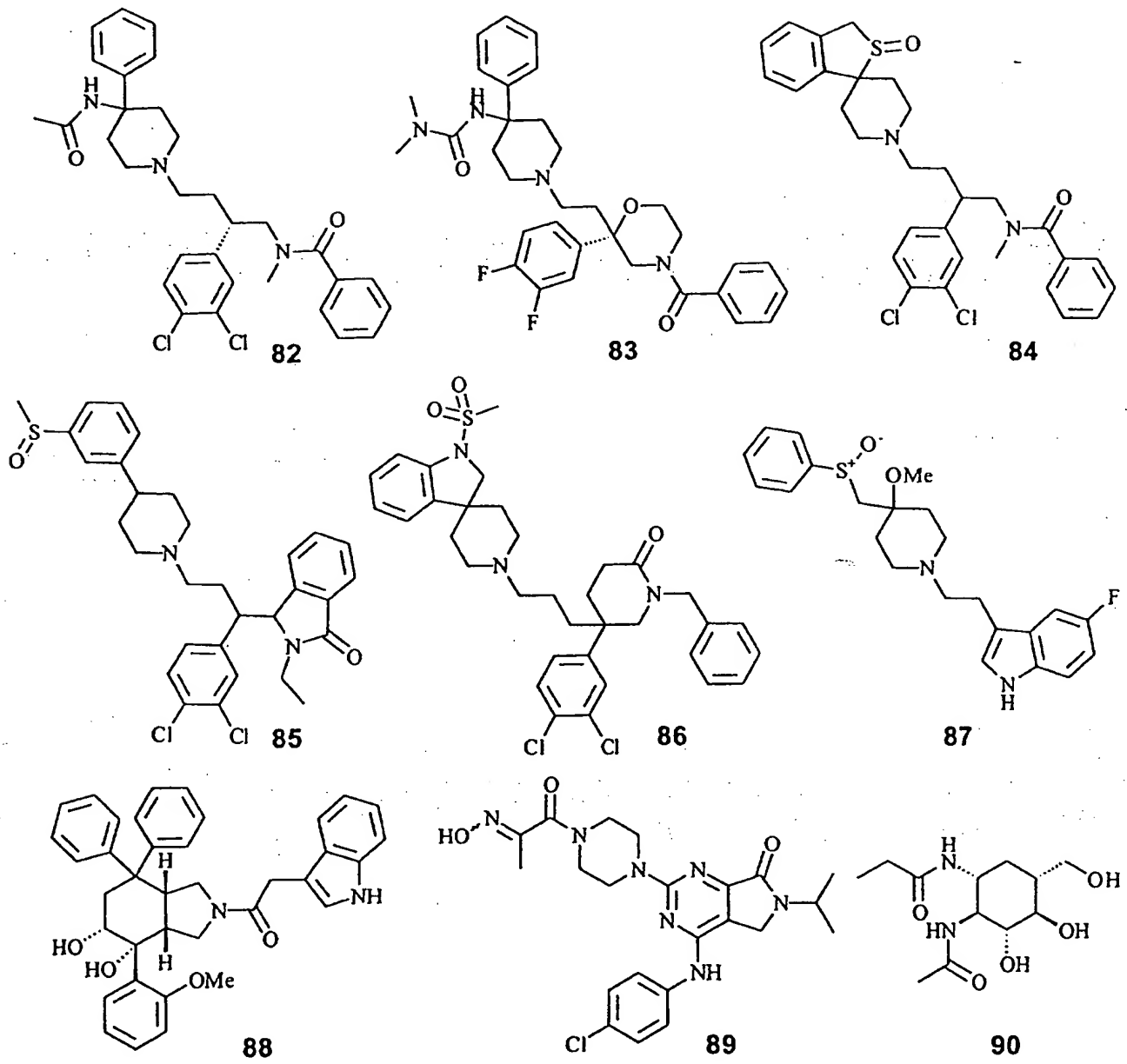


Fig. (28). NK_2 antagonists.

requirements for NK_1 receptor binding, thereby assigning the (*aR*,9*R*)-isomer as the active conformation [183].

Dual NK_1/NK_2 Antagonists

Since the release of SP and NKA causes mucus secretion, airway constriction, and plasma extravasation - typical clinical symptoms of asthma - it has been suggested to use dual NK_1/NK_2 antagonists in the treatment of asthma [184].

Considering the structural requirements of Sanofi's NK_2-selective antagonist SR-48968 **82** (Fig. (28), see below), researchers at Yamanouchi Pharm. developed the spiro[isobenzofuran]piperidine YM-35375 **70** (Fig. (23)) with binding affinity towards the NK_2 receptor with an IC_{50} value of 84 nM and an IC_{50} value 710 nM for NK_1, respectively. Further, it shows inhibitory activity (ID_{50}=41 μg/kg, *i.v.*) against [β-Ala^8]NKA(4-10)-induced bronchoconstriction in guinea pigs [185]. Utilizing this new NK_1/NK_2 dual antagonist as lead compound a further spiro-substituted piperidine analogue, YM-44778 **79** (Fig. (27)), was developed, exhibiting potent antagonistic activities against the NK_1 (IC_{50}=82 nM) and NK_2 (IC_{50}=62 nM) receptors in isolated tissues [185], respectively.

Based on L-tryptophanebenzyl esters, Qi *et al.* reported on the synthesis of two compounds **80**, and **81** with dual NK_1/NK_2 receptor affinity (Fig. (27)) [186].

80 contains a 4-spiroindano piperidine and shows dual NK activity combined with slightly improved NK_2 activity (IC_{50}=56 nM (*h*NK_1), IC_{50}=27 nM (*h*NK_2)). Upon incorporation f a 4-spiroindolin sulfonamide, the balanced antagonist **81** was obtained (IC_{50} = 14 nM - NK_1; 24 nM - NK_2).

NK_2 Antagonists

NK_2 antagonists are of particular interest for the treatment of chronic diseases such as asthma, inflammatory bowel disorders, rheumatoid arthritis, pain, emesis, and psychiatric disorders [157].

The first NK_2 antagonist, SR-48,968 **82** (Fig. (**28**)), Saredutant, was described in 1992 [187]. This potent antagonist has been shown to inhibit the NKA-induced brochoconstriction in isolated human airways. Only recently, a study of van Schoor *et al.* have demonstrated that NKA-induced bronchoconstriction in asthmatics was significantly reduced with 100 mg Saredutant administered *p.o* [188].

Based on this prototype compound, a number of analogues emerged from different laboratories. SR-144,190 **83** (Fig. (**28**)) retains the phenylpiperidine moiety but contains an additional morpholine unit in order to introduce rigidity. Compared to the parent compound, it exhibits a similar pharmacological profile with increased bioavailability in the CNS [189].

Also Yamanouchi (YM-38336) **84** and Zeneca (ZD-7944) **85** (Fig. (**28**)) presented potent NK_2 antagonists based on the Sanofi lead structure (SR-48,968 **82**). ZD-7944 **85** [190], showing a K_i value of 0.14 nM (MEL cells), still retains the phenylpiperidine entity, while YM-38336 **84** [191] has been modified by introduction of a spiro-benzothiophene residue in position 4 of the piperidine. YM-38336 **84** shows potent NK_2 inhibitory activity against [β-Ala^8]NKA(4-10)-induced bronchoconstriction in guinea pigs, demonstrated by an ID_{50} value of 20 mg/kg, *i.v.* [192].

Harrison *et al.* reported on the development of selective NK_2 and NK_3 antagonists based on a common structural template, notably the NK_3-selective compound SR-142,801 **91** (Fig. (**29**), see below) [193]. Transfer of the carbonyl oxygen from an exocyclic to an endocyclic position on the piperidine ring led to two series of selective analogues, NK_2 and NK_3 antagonists, respectively [193]. An example of a potent NK_2 antagonist is given by compound **86** (Fig. (**28**)) which exhibits an IC_{50} value of 2.2 nM for the displacement of [^{125}I]NKA from the cloned human NK_2 receptor in CHO cells.

A number of preclinical nonpeptide NK_2 antagonists have been reported by GlaxoWellcome, Rhône-Poulenc Rorer and Zeneca, e.g. GR-159,897 **87**, RPR-106,145 **88** (related to the NK_1 antagonist RPR-100,893 **76**, (Fig. (**25**))), and ZM-253,270 **89** (Fig. (**28**)) [158], respectively.

Menarini used an interestingly rigid template for its selective NK_2 antagonists (K_i=2.5 nM) MEN-11420 **90**, Nepadutant, exhibiting improved *in vivo* potency and duration which is attributed to its rigid structure [194].

NK_3 Antagonists

The first selective nonpeptide NK_3 antagonist, SR-142,801 **91**, Osanetant, has been reported by Sanofi (K_i=0.21 nM, CHO cells) (Fig. (**29**)) [195].

Based on this structural template, Merck Sharp and Dohme elaborated a series of NK_2 and NK_3 antagonists, exemplified with analogue **92** (Fig. (**29**)), the corresponding congener of **86** (Fig. (**28**)).

SmithKline Beecham claimed NK_3 antagonists for the treatment of CNS diseases, pulmonary disorders and dermatitis [196]. Based on a quinoline core structure,

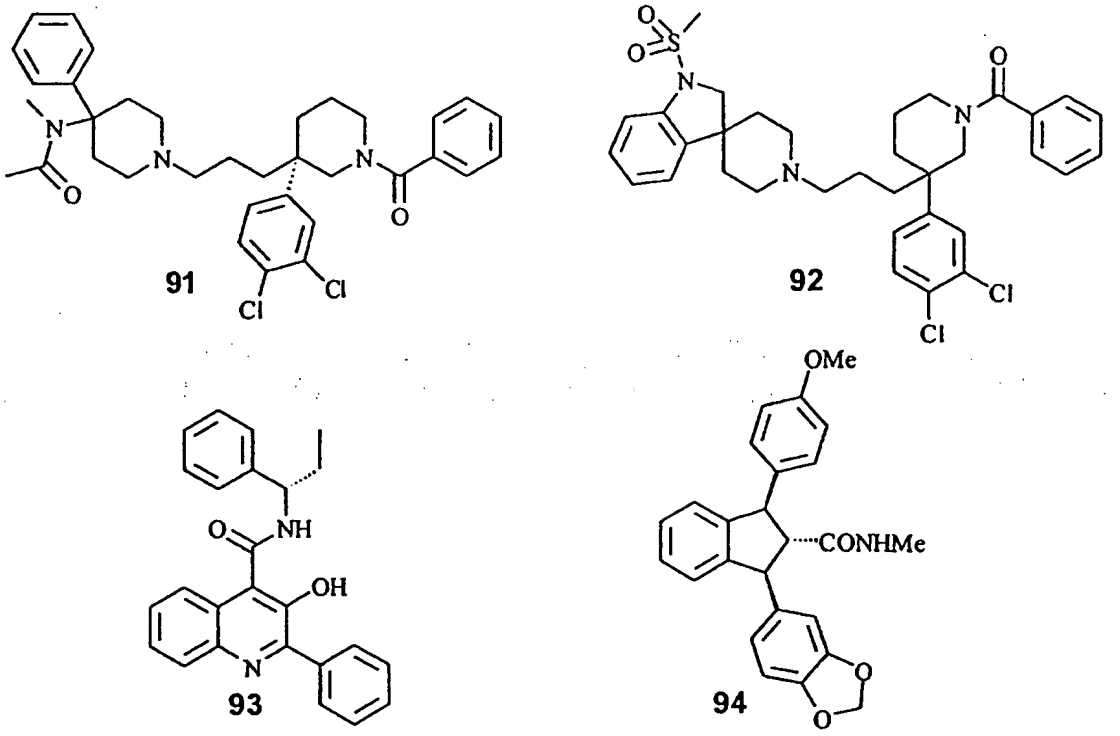


Fig. (**29**). NK_3 antagonists.

Giardina *et al.* developed SB-223,412 **93** (Fig. (29)) demonstrating high NK_3 activity (IC_{50}=1.2 nM, K_i=1.0 nM, CHO cells), weak NK_2 activity, and no affinity for other receptors including ion channels [197]. SB-223,412 **93** exhibits *in vitro* and *in vivo* oral and intravenous activity in animal models [198].

An entirely novel structure, **94** (Fig. (29)), has been claimed as NK_3 antagonist for the treatment of bronchitis, asthma, anxiety, Parkinson's disease and dermatitis [199]. Interestingly, this compound resembles strongly the indane carboxylic acids of SmithKline Beecham's ET antagonists.

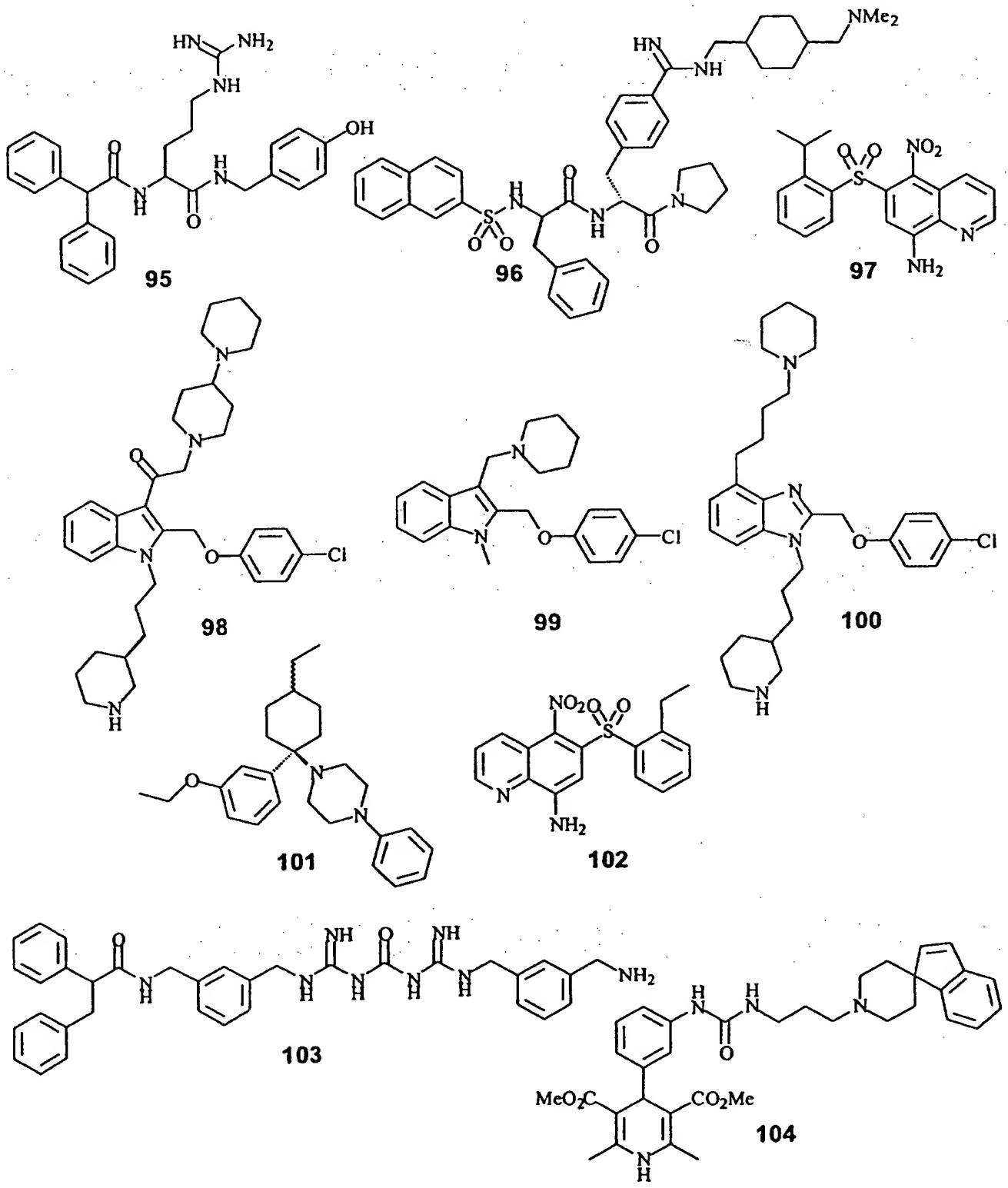


Fig. (30). Miscellaneous Y_1 antagonists.

Neuropeptide Y

Biomedical Significance

The 36-amino acid peptide neuropeptide Y (NPY, Table 1) was discovered in 1982 by Tatemoto *et al.* [200]. NPY is a member of the pancreatic polypeptide family, also including structurally related peptide YY (PYY) and pancreatic peptide (PP) [201]. NPY is widely distributed throughout the mammalian central and peripheral nervous system [202,203]. Interacting with its at least six receptor subtypes (Y_1-Y_6) it is involved in numerous physiological functions, e.g. food intake, blood pressure regulation, hormone secretion, sexual behaviour, and circadian rhythm [204-209]. Patent literature issued over the last ten years concentrate mainly on the inhibition of receptor-ligand interactions by low-molecular weight compounds in order to therapeutically interfere in mechanisms such as anxiety, appetite stimulation, obesity, alcohol intake, hypertension, and regulation of coronary tone [210]. As the Y_1 and Y_5 receptors are suggested to control feeding behaviour, they are believed to be the best target systems for developing antagonists as therapeutics for the treatment of obesity [204,211-213]. The Y_1 receptor, found in the peripheral and in the central nervous system (CNS), has been cloned in 1992 [214]. Its modulation may influence numerous physiological conditions including anxiety, diabetes, obesity, or appetite disorders. Most recently, the Y_5 receptor has been cloned and characterized to be involved in food intake regulation [212]. A review published by Ling in 1999 reports on the patent situation related to NPY antagonists [210]. In this contribution representative examples of potentially active nonpeptide NPY antagonists will be described according to their target receptors.

Y_1 Receptor Antagonists

A number of Y_1 antagonists (Fig. (30)) published over the last ten years show binding affinities in the nanomolar range, e.g. as BIBP3226 **95** (K_i=7.2 nM), SR120819 **96** (K_i=15 nM), PD160170 **97** (K_i=48 nM), and LY-357897 **98** (K_i=0.75 nM) (Fig. (30)) [215-218]. The best characterized Y_1 antagonist BIBP3226 **95** has been demonstrated to inhibit NPY-mediated vasoconstriction and pressure variations [215]. SR120819 **96** represents a dipeptide analogue containing a sulfonamide. This orally active antagonist incorporating a central arginine mimic (benzamidine in **96**) develops its potency in the 1,4-*cis*-disubstituted cyclohexyl ring by antagonizing NPY-mediated pressure responses [219].

Parke-Davis discovered a new and unique class of moderately potent but selective Y_1 antagonists by random screening of which PD160170 **97** is a representative compound. Eli Lilly described LY-357897 **98** from a series of trisubstituted indoles and benzimidazoles. Compound **99** (Fig. (30)) [220] showing a K_i value of 2.1 μM was discovered by a biased screening of the in-house library and served as lead structure in the subsequent SAR studies of the trisubstituted indole series. Consequent structure modification led to **98**, the most active analogue (K_i=0.75 nM), which, in (*S*)-configuration inhibits NPY-induced forskolin-stimulated cAMP release and intracellular Ca^{2+} release in the nanomolar range. The corresponding

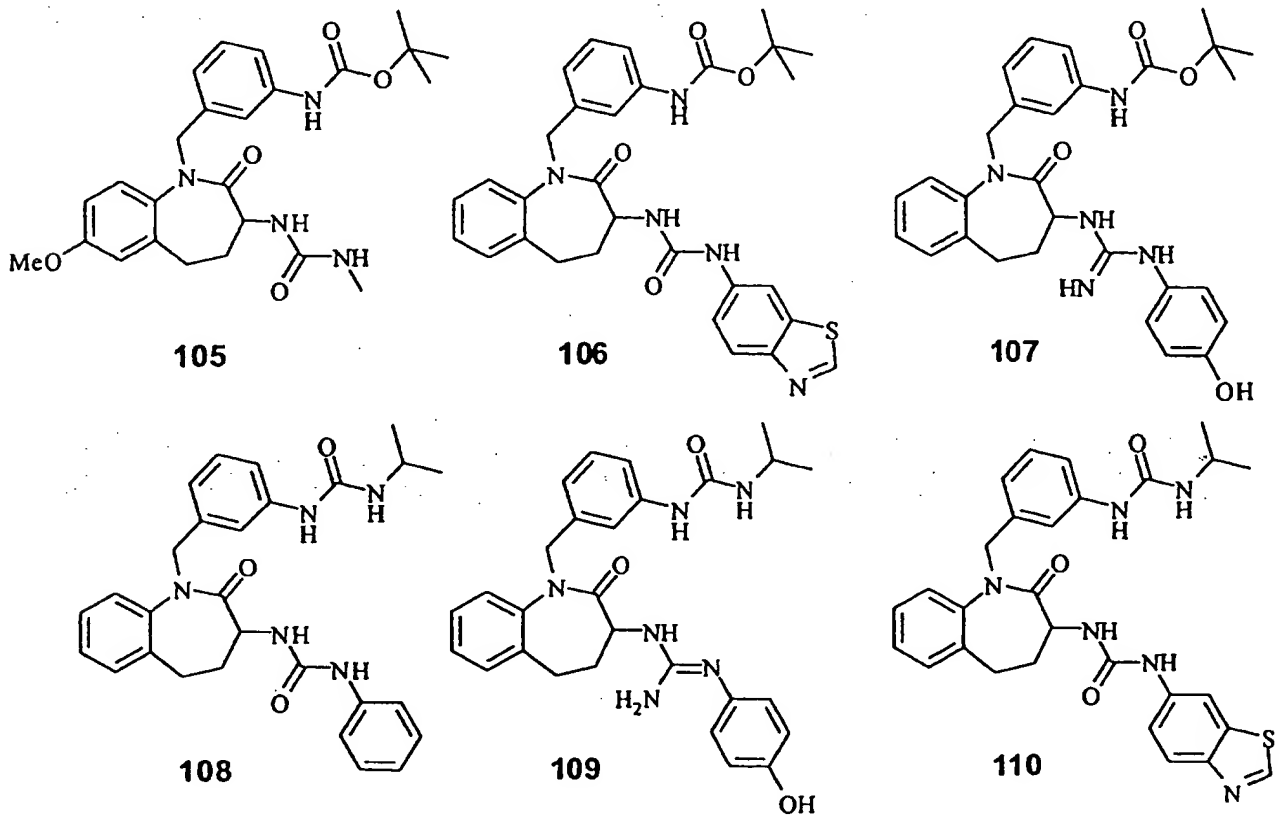


Fig. (31). Benzazepinone-type Y_1 antagonists.

benzimidazole series has also been investigated [221]. A representative example is given by compound **100** (Fig. (30)) which was obtained after systematic optimization of the N1- and C4-substituents of the benzimidazole scaffold. Compound **100** exhibits *in vitro* binding affinity on AV-12 cells expressing the human Y_1 receptor with a K_i value of 1.7 nM.

Pfizer claimed a series of piperazinyl-comprising compounds as Y_1-selective antagonists [222]. Analogue **101** (Fig. (30)) demonstrates an interesting activity profile by expressing a differentiated behaviour of the two conformers, i.e. *cis*- (IC_{50}=76 nM) and *trans*- (IC_{50}=525 nM) exposed ethyl substituent with respect to the phenylpiperazine substitutent of the cyclohexyl ring.

Warner Lambert filed compounds based on a quinoline scaffold that were claimed as Y_1 subtype selective antagonists. The 6-aryl-sulfonyl-quinoline analogue **102** (Fig. (30)) inhibits [^{125}I]PYY binding to the human Y_1 receptor with an K_i value of 48 nM [223].

Alanex Corp. claimed two series of compouds containing either an amidino-urea or a diamidino-urea core structure. A representative of the latter series is given by **103** (Fig. (30)) inhibiting the binding of [^{125}I]PYY to the Y_1 receptor in membranes derived from human neuroblastoma cell lines (SK-N-MC) with an IC_{50} value of 70 nM [224].

Bristol Myers Squibb's patents enclose two structurally related compound classes, i.e. phenyl-dihydropyridines [225] and phenyl-dihydropyrimidines [226]. In compound **104** (Fig. (30)) the *m*-substituted phenyl-dihydropyridine sidechain is terminated with a spiroindane, a structural element which is also found among other antagonists directed against numerous members of the peptide-binding GPCR superfamily.

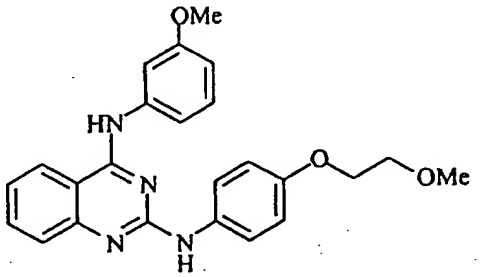
Murakami *et al.* [227] at Shionogi published a novel class of 1,3-disubstitued benzazepinones as potent and selective Y_1 antagonists. Based on the lead compound **105** (Fig. (31)) (K_i=1.5 μM) which emerged from a random screening approach, follow-up compounds **106** (K_i=160 nM) and **107** (K_i=39 nM) have been obtained (Fig. (31)).

Further optimization of the phenyl substituent in position 3 leading to analogue **108** as well as optimization of the substituent in position 3 of the 2,3,4,5-tetrahydro-1*H*-1-benzazepin-2-one, represented by congener **109** (Fig. (31)) resulted in an increase of the binding affinity towards 43 nM and 2.9 nM, respectively. Combination of the optimized structural features led to one of the most potent derivatives (**110**, Fig. (31)) which competitively inhibits specific [^{125}I]PYY binding to Y_1 receptors in human SK-N-MC cells with a K_i value of 5.1 nM. Although **110** also antagonizes the Y_1 receptor-mediated increase in cytosolic free Ca^{2+} concentration in SK-N-MC cells, it has not been evaluated *in vivo* because of its poor solubility in aqueous solution and poor oral bioavailability. Hence, it has been shown in binding assays with 17 receptors including the Y_2, Y_4, and Y_5 receptor that it binds selectively to the Y_1 receptor [227].

Y_5 Receptor Antagonist

Several patent applications have been filed by Novartis in 1997 [228-230] claiming diamino quinazolines as selective Y_5 antagonists. They were shown to inhibit NPY-induced Ca^{2+} increase in stable transfected cells expressing the Y_5 receptor. Analogue **111** (Fig. (32)) decreases food intake by 60% in 24 h food deprived rats after *i.p.* administration of 30 mg/kg.

In 1998 Banyu Pharm. [231,232] and Bayer [233] filed patents including aminopyrazoles, aminopyridines and an amide based core structure as Y_5 antagonists. The Banyu compounds **112** and **113** showed IC_{50} values for Y_5 binding of 8.3 nM and 4.1 nM, respectively [234], whereas the Bayer compound **114** binds with an IC_{50} value of 0.47 nM. Also this congener shows selective affinity for the Y_5 receptor compared to Y_1, Y_2, or Y_4 receptor subtypes (Fig. (32)) [234].



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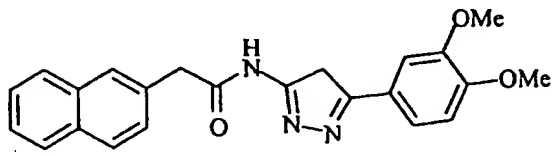

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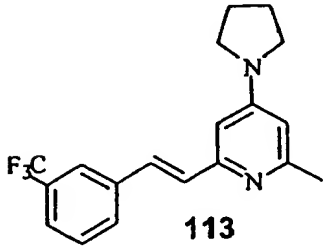

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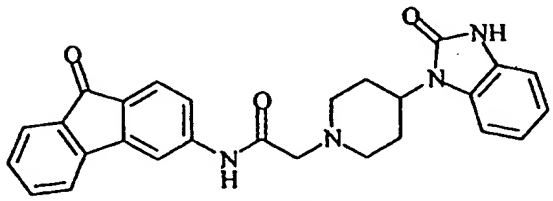


114

Fig. (32). Y_5 antagonists.

STRUCTURAL-BASED DRUG DESIGN

After having addressed the classical lead finding approach characterized by screening compound libraries with subsequent optimization, the complementary strategy of structure-based design will be highlighted, since this strategy is about to change the classical paradigm of "random *versus* rational" in favour of "random *goes* rational". Due to the fact that no high-resolution structure of any GPCR protein is available, all design attempts are still restricted on comparative analyses of structural features of biologically characterized low-molecular weight compounds which are interpreted in terms of steric and physicochemical complementarity to a hypothetical receptor binding site. Currently pursued GPCR research projects represent textbook examples for the fruitful combination of ligand-derived rationales that are incorporated into e.g. the design of combinatorial chemistry programs with the aim to direct resulting libraries more efficiently to the target class of interest, rather than attempting to explore systematically the infinite universe of molecular diversity. In the following, a few representative research efforts will be introduced that clearly attempt to change the mainstream of classical lead finding programs in favour of knowledge-based approaches.

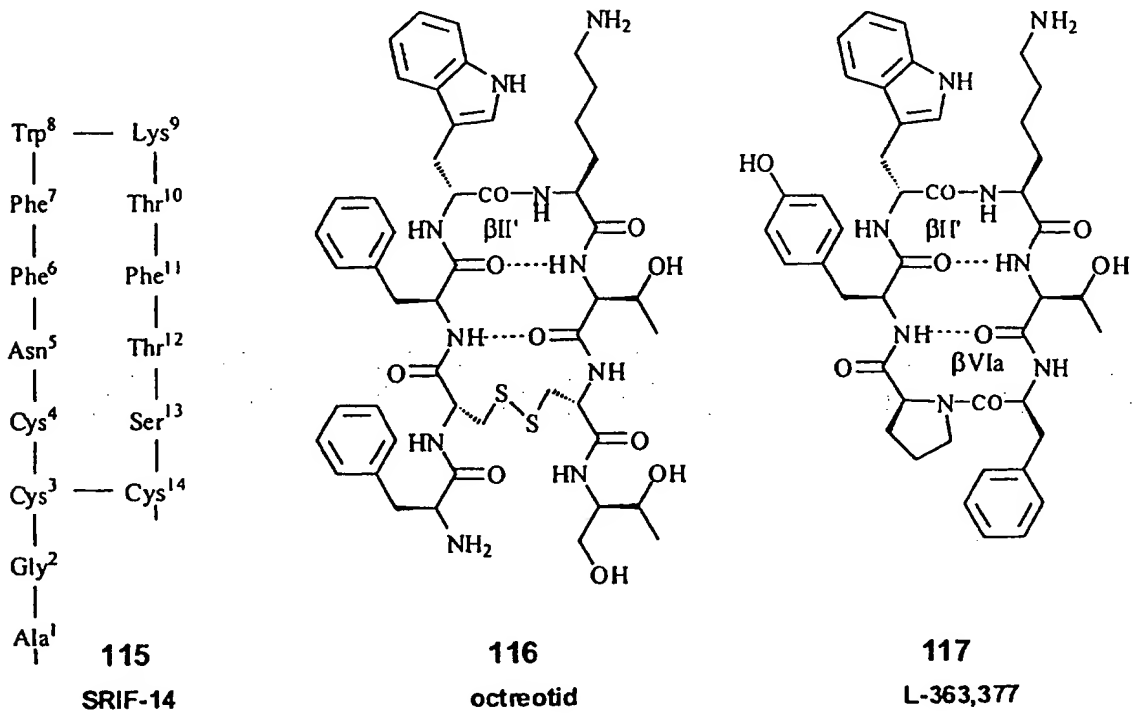
Somatostatin

Somatostatin (Somatotropin Release-Inhibiting Factor, SRIF) (Table 1) was discovered because of its inhibitory effect on growth hormone secretion. The peptide hormone which exists in two biologically active forms, the 14 amino acid form (SRIF-14) and the 28 amino acid form (SRIF-28), acts as a neuromodulator [235].

Five receptor subtypes for somatostatin (sst_1-sst_5) have been cloned and characterized from human tissue [236]. Apart from its pivotal role as neuromodulator within the central nervous system (CNS), somatostatin alters the secretion of growth hormone (GH), insulin, glucagon, pancreatic enzymes, and gastric acid [237-240]. Consequently, analogues of somatostatin emerged as interesting tools in the treatment of disorders linked to the above mentioned physiological functions. Somatostatin agonists may therefore be used for the treatment of acromegaly, diabetes, cancer, rheumatoid arthritis, and Alzheimer's disease. Especially sst_2-selective agonists emerged as useful candidates for the treatment of acromegaly, retinopathy, and diabetes [241,242].

The area of somatostatin agonist and antagonist research is a textbook example for indirect drug design utilizing ligand-derived structural rationales for design purposes. In the beginning of the 1990's numerous design projects were pursued aimed to replace the peptide scaffold of the pharmacophoric portion of somatostatin (SRIF-14) yielding a variety of moderately active, chemically diverse compounds. More recent lead finding programs employ the highly efficient technology of combinatorial chemistry for rapid modification of promising hits culminating in subtype-selective high-affinity binding compounds from a series of designed libraries. A brief overview of both, the rational design of single somatostatin-based peptidomimetics as well as the combinatorial chemistry-based approaches for lead identification and optimization will be given after a short description of the somatostatin-relevant pharmacophore hypothesis.

The tetradecapeptide SRIF-14 **115** (Fig. (33)), one of the widely distributed active forms of somatostatin, is believed



115 SRIF-14 **116** octreotid **117** L-363,377

Fig. (33). Conformational preferences of somatostatin-derived peptide analogues.

to adopt a two-stranded β sheet conformation induced by a β turn encompassing Phe^{7}-Trp^{8}-Lys^{9}-Thr^{10}, and the disulfide bridge between Cys^{3} and Cys^{14}, respectively (Fig. (33)). The conformation is further stabilized by the transannular H-bonding pattern typical for antiparallel sheet structures. From numerous sequence- and structure-activity studies it turned out that the primary pharmacophore consists of the β turn forming residues Phe^{7}-Trp^{8}-Lys^{9} and an additional lipophilic binding element reminiscent to Phe^{6}/Phe^{11} [243].

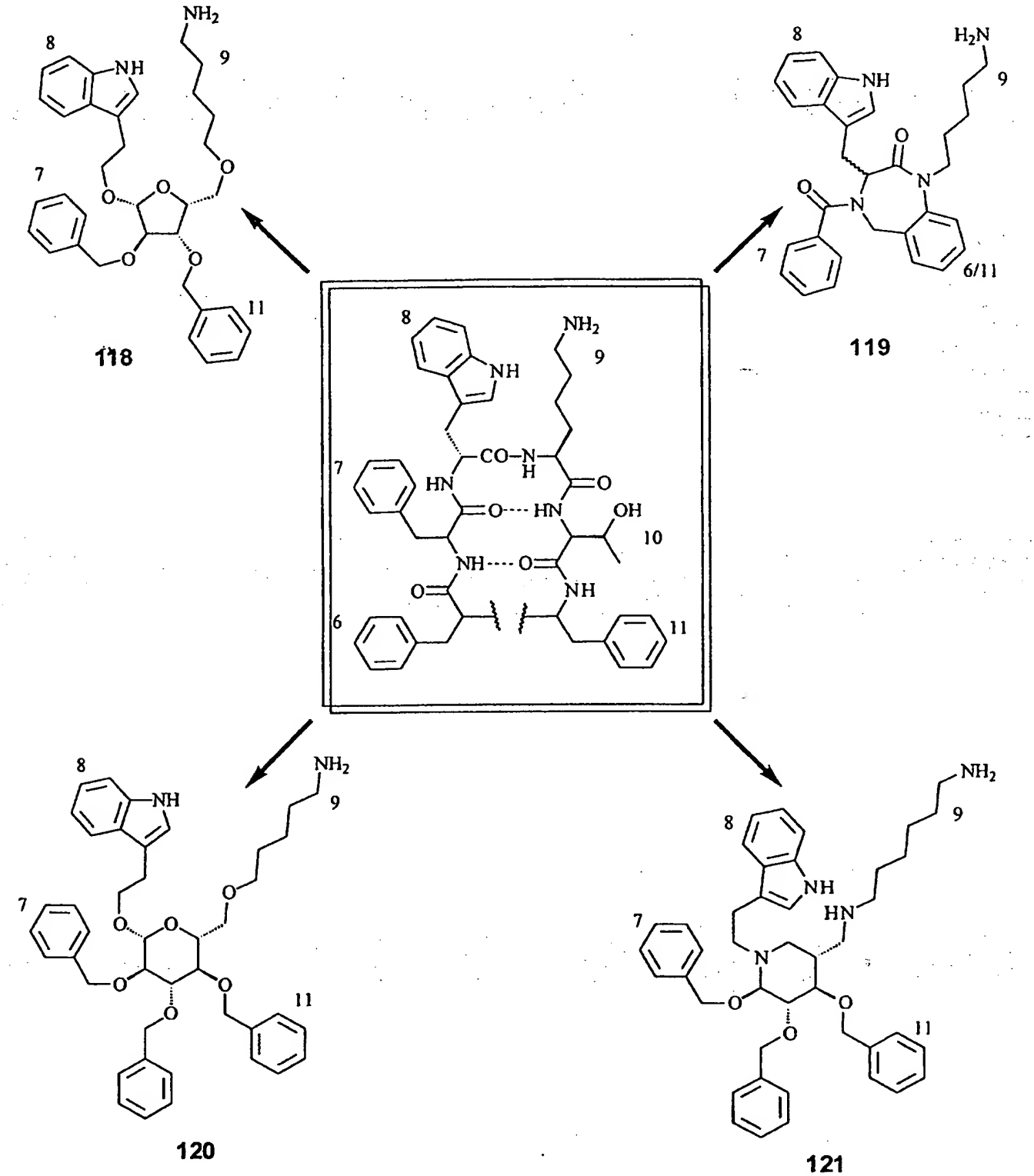


Fig. (34). Peptide conformation-derived non-peptide somatostatin antagonists. The numbering scheme refers to that of SRIF-14 (see Fig. (33)).

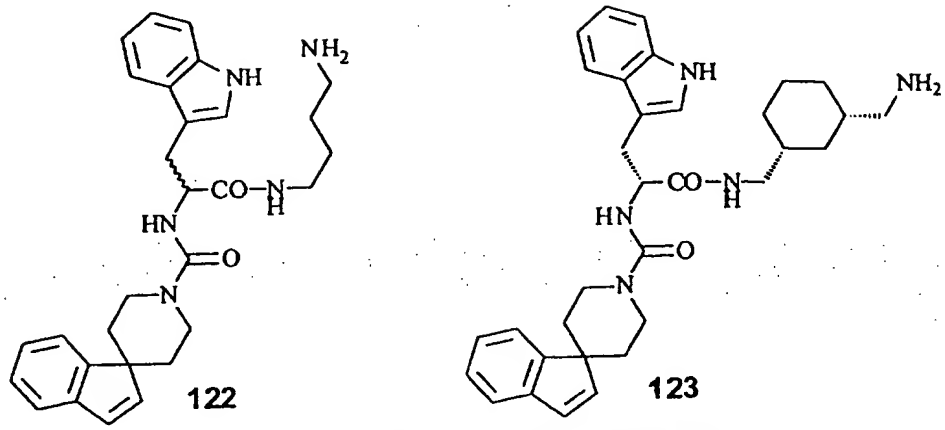


Fig. (35). High-affinity *h*sst$_2$ antagonists derived by screening and subsequent optimization.

The experimentally derived conformations of the metabolically more stable peptide analogues, e.g. octreotide (Sandostatin®) **116** [244,245] or L-363,377 **117** [246,247] not only prove the pharmacophore hypothesis, but were further used as template structures underlying a series of rational design attempts (Fig. (33)). In 1992, researchers at Sandoz designed a tetra-substituted xylofuranose derivative **118** (Fig. (34)) positioning the sidechains of Phe^7-Trp^8-Lys^9 at its C-2, C-3, and C-5 atoms, while the benzyloxy group attached to C-3 resembles the aromatic sidechain of Phe^{11}, respectively (Fig. (34)) [248].

The xylose derivative **118** displaced radio-labelled octreotide **116** from its receptor with an IC_{50} of 23 μM. Even though the mutual steric fit of the xylose-based mimic and the somatostatin structure was reasonable, the compounds displayed only moderate affinity which was attributed to the loss of considerable conformational entropy during receptor binding. Consequently, the design strategy at Sandoz was directed towards more rigid compounds based on nonpeptide scaffolds. For the purpose of substituting the peptide backbone of SRIF-14 within the β turn portion the privileged structure of the 1,4-benzodiazepinone was employed from which the pharamcophoric groups could radiate into the periphery [249]. The resulting nonpeptide tetrapeptide-mimetic **119** (Fig. (34)) was designed to account for the sidechains of Phe^7-Trp^8-Lys^9 by the appropriate substituents, while the aromatic ring of the benzodiazepine

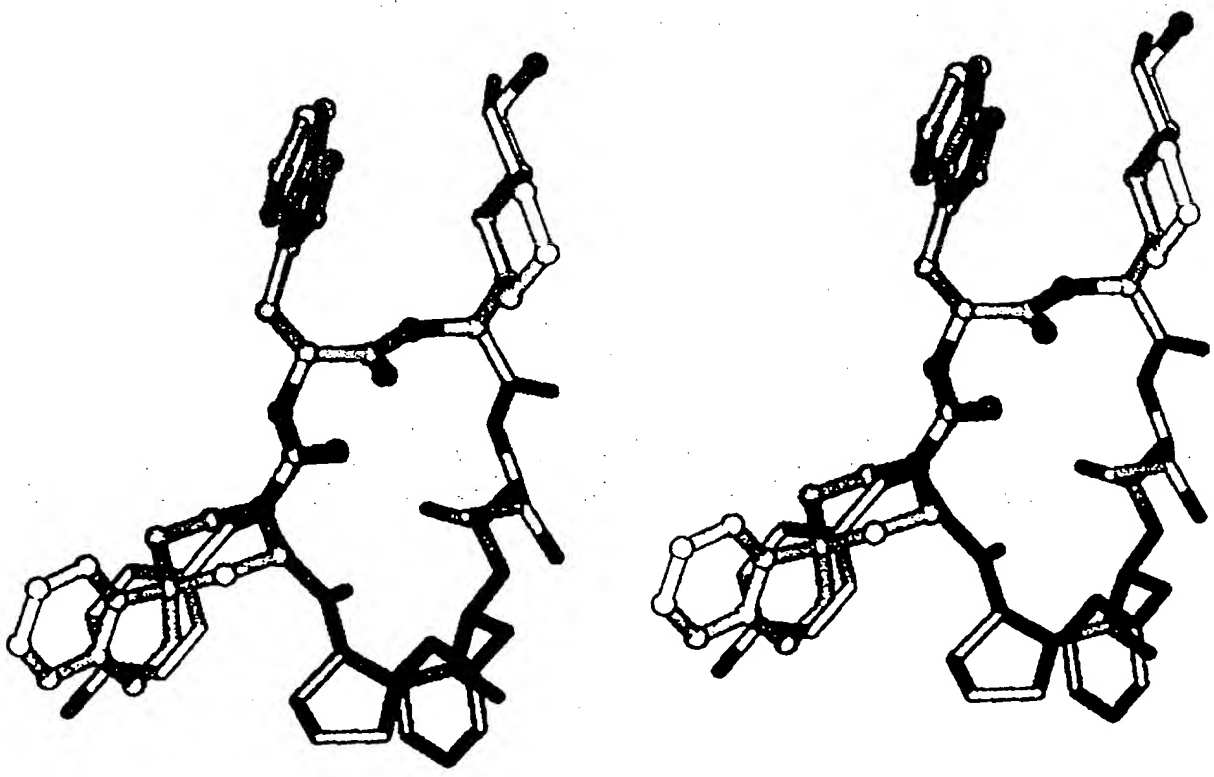


Fig. (36). Side-by-side stereo presentation of the structural overlay of **123** (ball-and-stick mode) onto the experimentally-derived conformation of **117** (stick-mode).

core was believed to mimic the additional lipophilic element referring to Phe^{6}/Phe^{11}, respectively. However, the racemic mixture of **119** (benzodiazepinone) showed an IC_{50} of 7 μM, and even after separation, the L- and D-Trp containing benzodiazepinone displaced the radioligand with IC_{50} of only 6.5 μM and 8.2 μM, respectively.

Similar affinities in the low micromolar range were obtained with peptidomimetics based on β-D-glucose scaffolding described by Hirschmann and Nicolaou at the end of the 80's and beginning of the 90's [250]. Molecular modeling studies carried out on the 3D structures of SRIF-14 **115** and analogues of L-363,377 **117** suggested that substituents at C-2, C-1, and C-6 of a β-D-glucose template resemble the orientational pattern of the β turn-forming amino acids of the somatostatin-derived peptides. The corresponding penta-substituted glucose **120** (Fig. (34) showed an IC_{50} of 15 μM.

In 1996, researchers from Rhône-Poulenc Rorer published a similar approach of *de-novo* designed peptidomimetics employing aza-sugar-based templates for the spatially controlled orientation of the pharamcophoric amino acid sidechains [251]. Independent of ring size and substitution

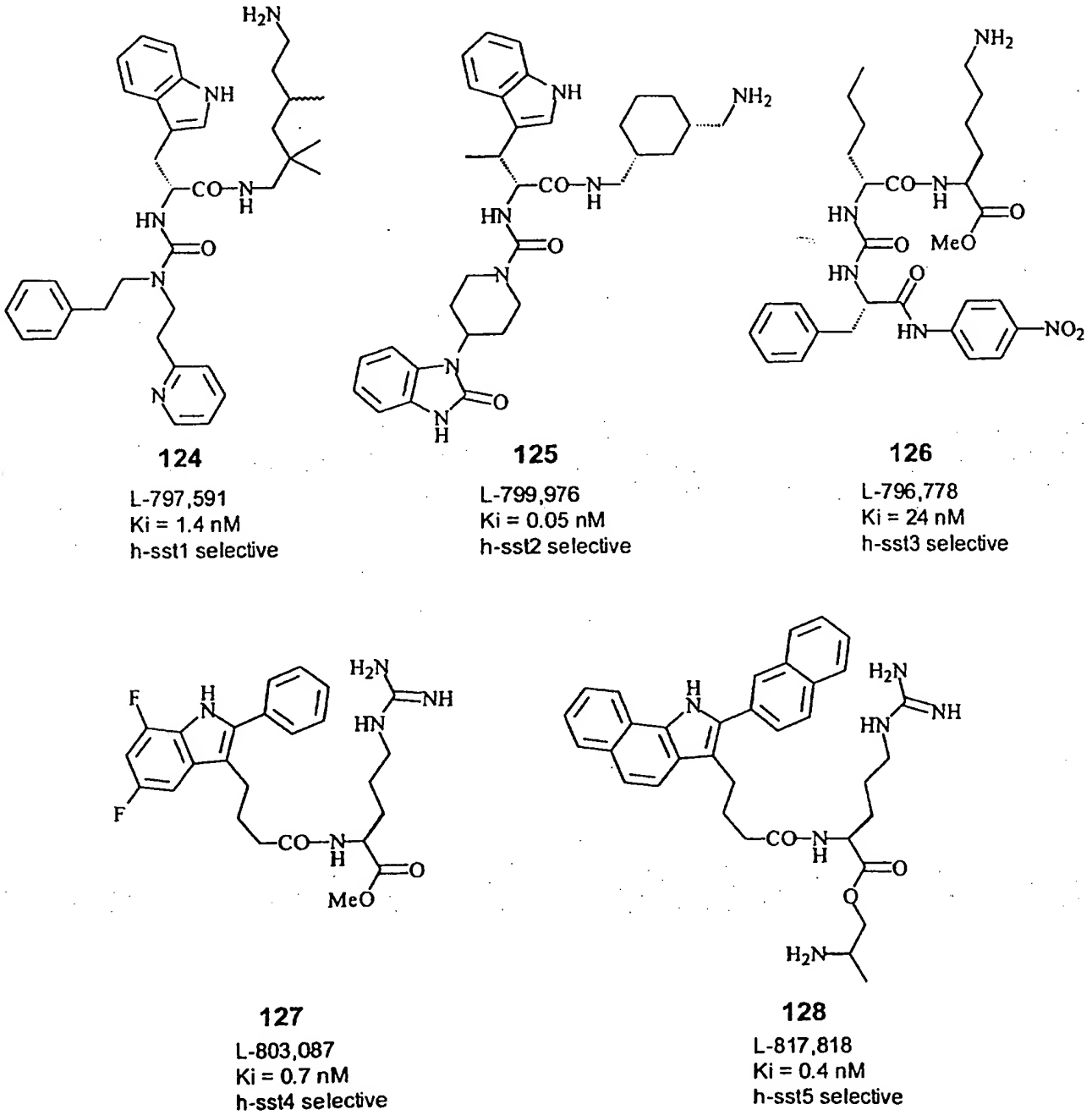


Fig. (37). For each somatostatin receptor subtype (*hsst*$_1$-*hsst*$_5$) highly selective compounds emerged from rationally designed combinatorial libraries.

pattern, all analogues showed weak affinity with IC_{50} values in the range of 10-15 μM (see for example **121**, Fig. (34)).

Over the last two years, scientists at the Merck Research Laboratories conducted a comprehensive program aimed to identify subtype-selective peptidomimetic compounds for each somatostatin receptor subtype (sst_1-sst_5) by following a rational design strategy using a combination of classical medicinal chemistry with modern combinatorial chemistry techniques [252-256]. The primary lead, L-264,930 **122** (Fig. (35)), that initiated that combined approach, was identified by a virtual screening of the Merck sample collection. The 3D structure of the cyclic hexapeptide L-363,377 **117** (Fig. (33)) served as spatial probe in that a geometric pattern, describing the arrangement of the pharmacophoric groups, was derived by means of molecular modeling. After similarity searches, in which the sidechains of residues Tyr^7-Trp^8-Lys^9 were given priority for the pharmacophore definition, L-264,930 **122** was uncovered with submicromolar affinity for the *h*sst_2 receptor.

This compound became the primary focus for medicinal chemistry and combinatorial chemistry at Merck. By constraining the floppy diamine chain with a 1,3-bis-aminomethyl-cyclohexane moiety the compound was optimized to yield L-054,264 **123** (Fig. (35), Fig. (36)) with an IC_{50} of 1.6 nM for the *h*sst_2 receptor and a more than 1000-fold selectivity over all other somatostatin receptor subtypes.

Simultaneously, L-264,930 **122** served as lead structure for a targeted combinatorial library. For library design the lead was dissected into three components, notably the central α-amino acid, the *C*-terminal blocking diamine, and the *N*-terminal blocking bulky urea-attached amine. The initial library was based on 20 α-amino acids, that were mainly analogues of Trp or carried modified aromatic sidechains. Additionally, 20 diamines were chosen in which the spacing between the two nitrogens varies between four and six atoms, also encompassing different ring topologies. The amine collection comprised 79 different entities that were biased towards piperidines and piperazines containing additional aromatic rings, so-called "privileged structures". A solid-phase mix-and-split protocol was used to synthesize more than 130000 compounds in complex mixtures that demanded a deconvolution strategy. After several rounds of iterative optimization employing classical analoging as well as follow-up libraries, five compounds **124** - **128** emerged with the desired activity and selectivity profile, in that each compound is highly selective for a distinct somatostatin receptor subtype (Fig. (37)).

This program impressively demonstrates the impact of an intelligent combination of structural rationales derived by comprehensive molecular modeling with the synthetic efficiency of current combinatorial chemistry techniques for lead finding attempts within modern medicinal chemistry.

A further example of a peptidomimetics-based library employing structure rationales for identification of subtype-selective somatostatin analogues was published recently by J. Ellman and co-workers (Fig. (38)) [257]. By decoration of a medium-sized heterocyclic β turn mimic with the Trp- and Lys-sidechain in positions *i*+*1*-*1*+*2* and vice versa, together with an additional amine building block in *i*+*3*, a remarkably small library of only 172 entities (22 amines, D/L-Trp-D/L-Lys, D/L-Lys-D/L-Trp) uncovered a *h*sst_5-selective compound **129** with an IC_{50} of 87 nM.

Bradykinin

Researchers at Sterling Winthrop considered angiotensin-converting-enzyme (ACE) inhibitors as templates for the design of BK B_2 receptor antagonists [258], since ACE degrades both, angiotensin II (AII) and BK by cleaving the Pro^7-Phe^8 amide bond. Therefore, an ACE inhibitor was considered to display properties or conformational similarities to BK, thus establishing a pharmacophoric link between ACE and BK receptors in that both macromolecules recognize similar steric and physicochemical features. In order to test this hypothesis, the ACE inhibitor Quinapril

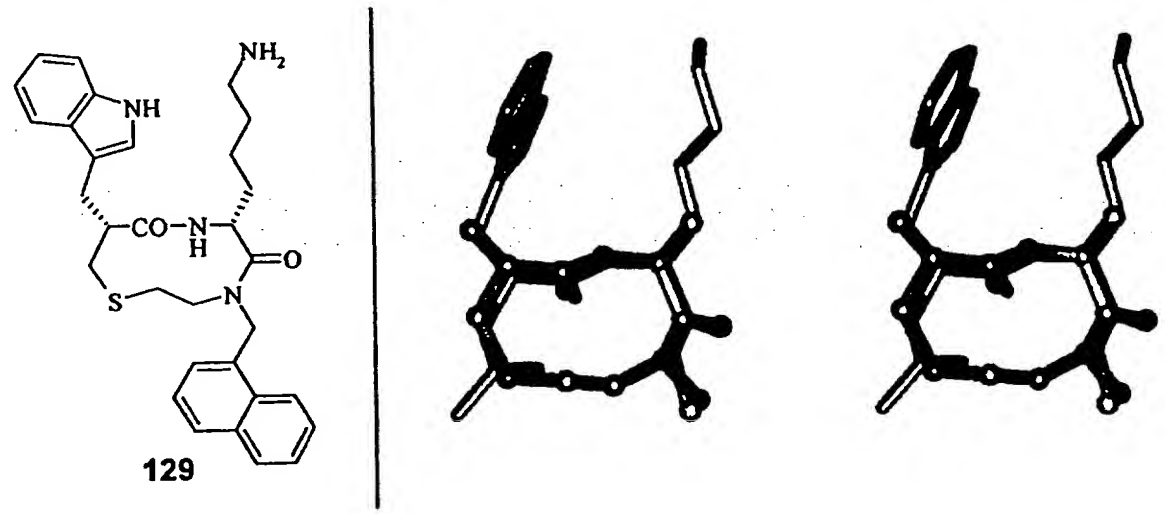
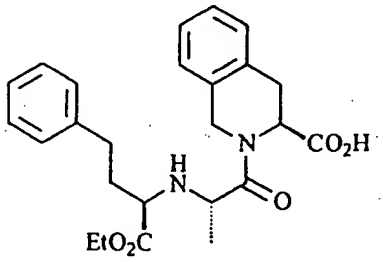


Fig. (38). Left: *h*sst_5-selective compound derived from a β turn-templated library; right: side-by-side stereo presentation of the structural superposition of the β turn mimic (ball-and-stick mode) onto the βII' turn portion of **117** (stick-mode).

130 (Fig. (39)) [259] was chosen as template for the design and synthesis of a series of *homo*Phe-Tic (Tic: tetrahydrisoquinoline) containing compounds. The diastereomeres of **131** (Fig. (39)) exhibit binding affinities in the micromolar range ($K_i \approx 1$ μM) in [^{3}H]BK binding studies with human IMR-90 fetal lung fibroblasts.



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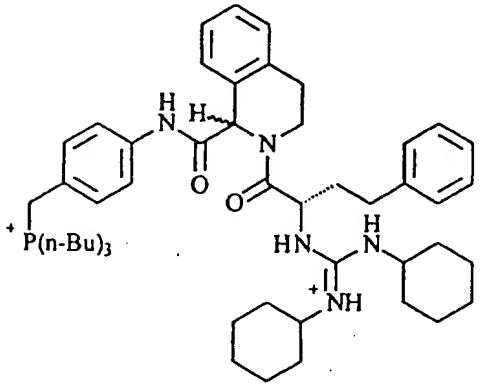


131

Fig. (39). Quinapril (**130**) served as template for the design of BK antagonists (e.g. **131**).

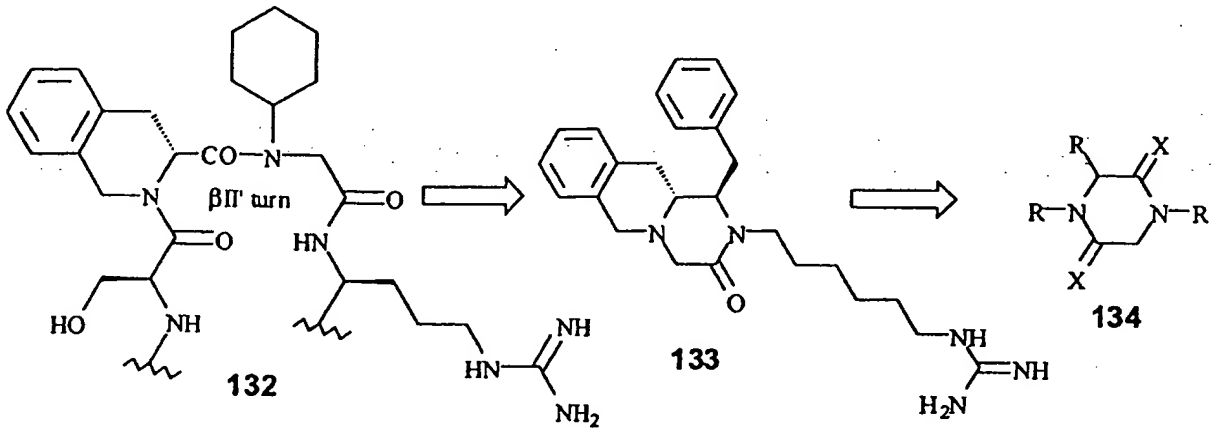
Goodfellow *et al.* [260] followed a different approach in that they established a library based on a β turn template, CP-0597 **132** (Fig. (40)) [261] which is a peptidic B_1/B_2 antagonist containing D-Tic and *N*-Chg (Chg: N-cyclohexylglyine) in *i+1* and *i+2* position of a βII' turn. Starting from that structural rationale, the peptidomimetic CP-2055 **133** (Fig. (40)) was generated. Based on the 1,4-piperzine scaffold a combinatorial library has been designed to produce approximately 2500 *rationally directed diverse analogues* (RDDA), **134** (Fig. (40)).

This process led to the discovery of nonpeptide B_2 antagonists serving as lead compounds for traditional optimization. While the parent peptidic analogue CP-0597 **132** shows an IC_{50} value of 0.33 nM, CP-2055 **133** exhibits an IC_{50} value of about 55 μM on a cloned human B_2 receptor. CP-2458 is a further a member of the designed library **134** and inhibits human B_2 receptor binding (IC_{50}=4.1 μM) and BK-stimulated Ca^{2+} flux in human fibroblasts (IC_{50}=19 μM). Unfortunately, the chemical formula of the compound is not given explicitly in the publication.

Based on two structural templates (i) a cyclic hexapeptide BK antagonist **135** [262] and (ii) the nonpeptide BK antagonist WIN-64338 **43** (Fig. (41)) [129], Dankwardt *et al.* [263] designed nonpeptide B_2 antagonists. While the hexapeptide served as structural template for the positioning of relevant functionality, WIN-64338 **43** served as rigid scaffold for the design of a series of naphthylalanine containing derivatives, none of which showed improved affinity for the B_2 receptor when compared to WIN-64338 **43** (K_d = 44 nM. Substitution of the phosphonium group against the corresponding ammonium moiety resulted in a two-fold decrease in affinity for the B_2 receptor. However, the proposed structural superposition of the cyclic hexapeptide **135** with the blocked amino acid derivative **43** provided a pharmacophore hypothesis that enabled Dankwardt and coworkers to design moderately active compounds and might serve as structural blueprint for further design attempts[263].

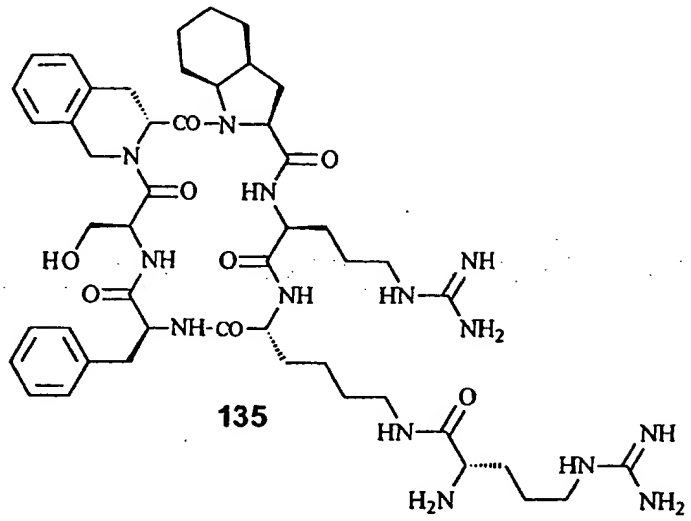
Neurokinin

The structural feature of a reverse β turn has emerged to a general design principle underlying a variety of GPCR antagonist projects. β truns play an important role in recognition phenomena as documented e.g. for somatostatin and NKA which bind to their receptors in a proposed β turn conformation. Therefore Horwell *et al.* [264] at Parke-Davis



132 **133** **134**

Fig. (40). Design strategy of BK antagonists following the "*rationally directed diverse analogues*" approach.



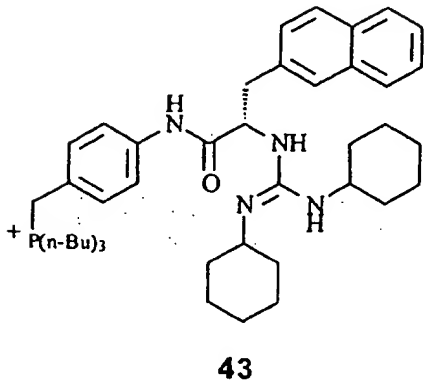


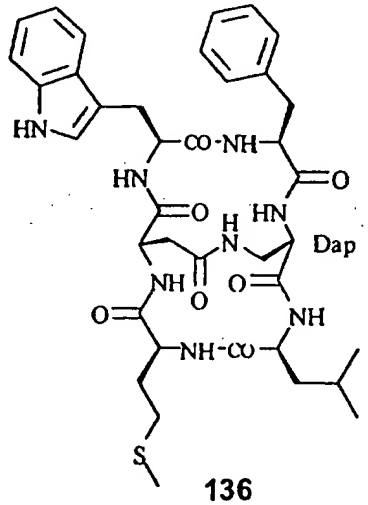
Fig. (41). Rationally designed BK antagonists.

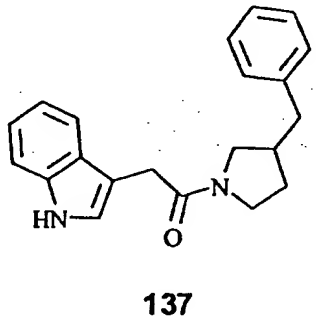
decided to employ β turn mimetics for the design of compounds with affinity for the NK_2 receptor. Starting from the x-ray structure of MEN-10627 **136** (Fig. (**42**)) [265], a cyclic hexapeptide displaying high NK_2 affinity, a pyrrolidine-based Trp-Phe dipeptide mimetic **137** has been designed (Fig. (**42**)).

The Trp-Phe dipeptide scaffold mimics the Trp-Phe fragment in the central portion (*i+1, i+2*) of a βI turn within the cyclic hexapeptide which folds into a βI/βII turn conformation. Although the indole and benzyl sidechains of both compounds superimpose satisfactory, **137** did not show significant NK_2 receptor affinity. The lack of affinity has been attributed to the misfit of the dipole moments of both molecules. In order to address this problem in more detail, a further Trp-Phe dipeptide mimetic **138** (Fig. (**42**)) has been designed by computer-assisted molecular modeling identifying a 2-azabicyclonorbornan spacer to be more favourable compared to the pyrrolidine (Fig. (**43**)).

Comparison of the binding affinities revealed that the conversion of the hexapeptide to a dipeptide unit results in the loss of high binding affinity (MEN10627 **136**: IC_{50}=0.079 nM (NK_2); **137**: IC_{50}=14% @ 10 μM (NK_2); **138**: IC_{50}=31% @ 10 μM (NK_2)) studied by displacement assays with [^{125}I]NKA in hamster urinary bladder. On the other hand, [^{125}I]BH-SP displacement from NK_1 in human IM-9 cells of MEN-10627 **136** (IC_{50}=0.8 μM) is retained by **137** and **138** with IC_{50} values of 3.7 μM and 6.7 μM, respectively. Interestingly, the dipeptide mimetics exhibit some binding affinity to human NK_3 receptors stably expressed in CHO cells shown by replacement of [^{125}I]-[MePhe^7]NKB (**137**: $IC_{50}(NK_3)$=3.5 μM; **138**: $IC_{50}(NK_3)$=35% @ 10 μM) while the parent hexapeptide exhibited no NK_3 affinity at all.

Only recently, Porcelli *et al.* [266] presented the design of a SP antagonist based on a cyclic pentapeptide with the chirality sequence following a $D^1L^2D^3D^4L^5$ pattern. The





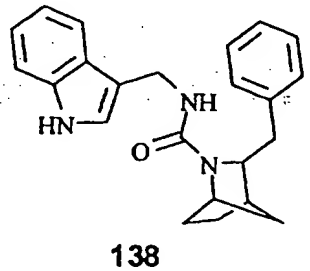


Fig. (42). Peptide structure-derived rationales were used to design non-peptide NK antagonists (Dap: 2,3-diaminopropanoic acid).

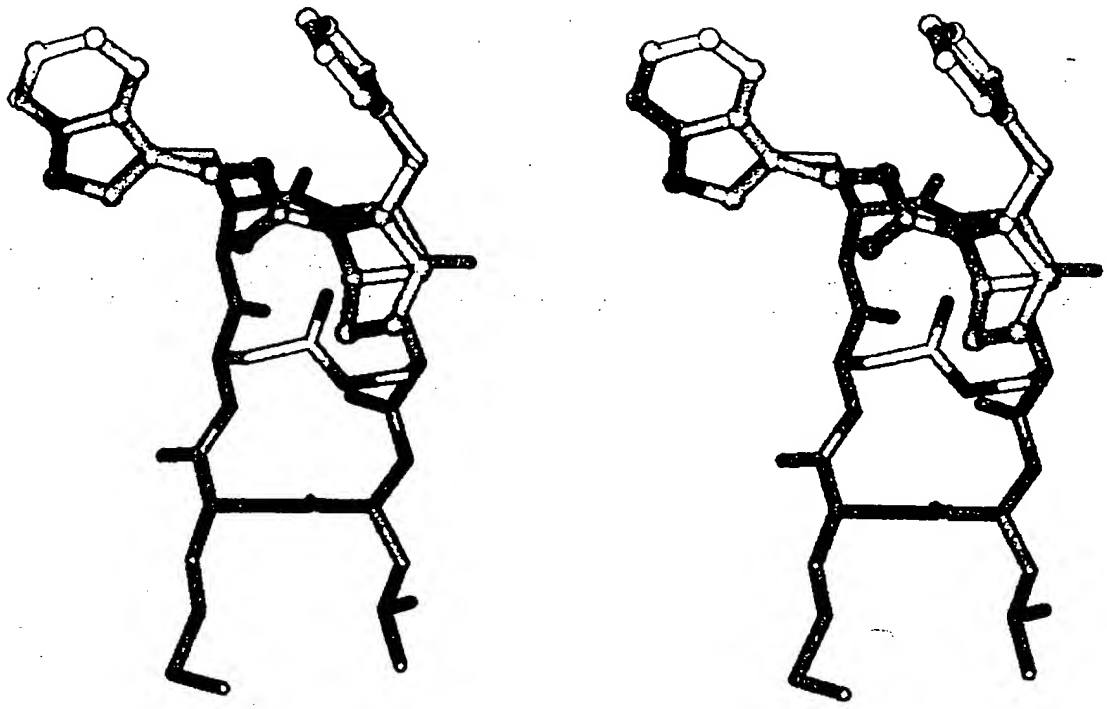
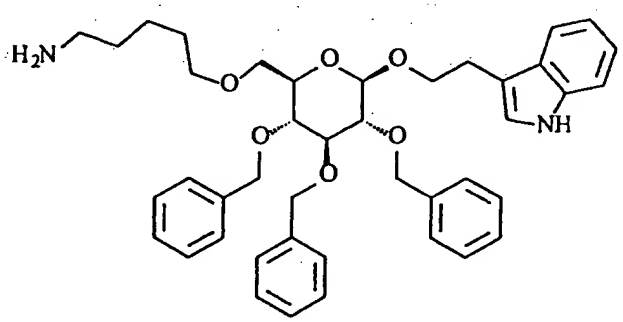


Fig. (43). Side-by-side stereo presentation of the structural overlay of 138 (ball-and-stick-mode) onto the x-ray structure of 136 (stick-mode) within the turn corresponding portion.

authors suggest this scaffold as a generic template to design antagonists also for other members of the GPCR family. This suggestion is the logical consequence of the fact that among potent GPCR antagonists the same unique skeleton is found among other representatives of antagonists for peptide-binding GPCRs, e.g. the natural pentapeptide BE-18257B (*cyclo*-(D-allo-Ile-Leu-D-Trp-D-Glu-Ala-)) and its synthetic analogue BQ-123 (*cyclo*-(D-Val-Leu-D-Trp-D-Asp-Pro-)) [267], a prominent ET_A antagonist. Both cyclic pentapeptides follow the chiral sequence pattern of DLDDL. The solution structure of BQ-123 [268] exhibits a typical $\beta II/\gamma_i$ turn arrangement characteristic for this class of molecules. Based on the same structural template, Porcelli *et al.* designed a SP antagonist, ITF-1565 (*cyclo*-(D-Trp^1-Pro^2-D-Lys^3-D-Trp^4-Phe^5-)) which inhibits NK_1-mediated SP-induced contraction of the rabbit caval vein. ITF-1565 only shows modest NK_2 activity and was inactive in ET_A assays. ITF-1565 exhibits a $\beta II/\gamma$ turn arrangement with Pro^2 in *i+1* and D-Lys^3 in *i+2* position of the β turn and Phe^5 in the central position of the γ turn. Interestingly, the authors succeeded to superimpose the sidechain functionalities of D-Trp^4, Phe^5 and D-Trp^1 within ITF-1565 well onto the indole and benzyl rings within a β-D-glucose derived SP antagonist **139** (Fig. (44)).



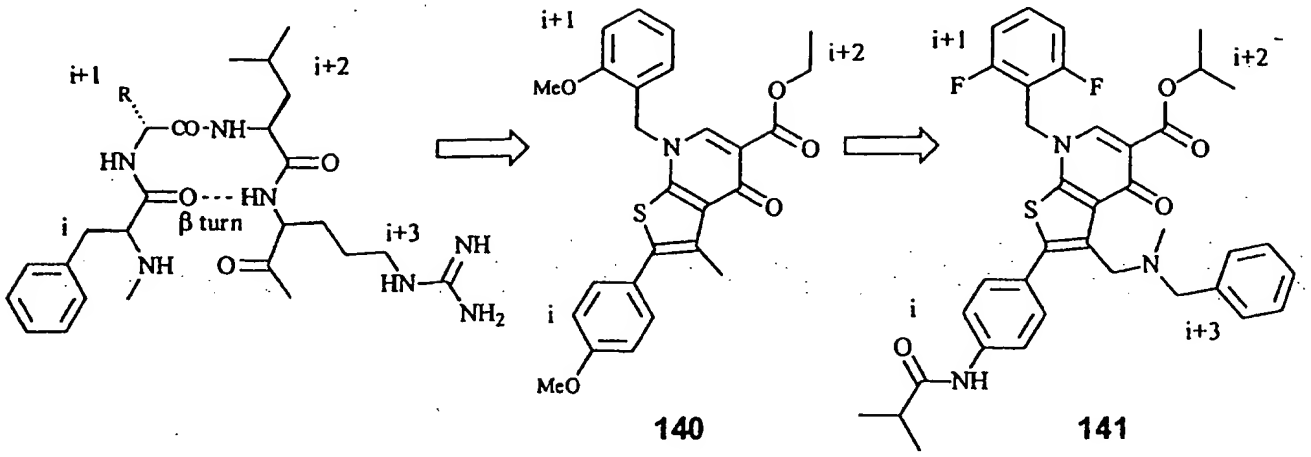
139

Fig. (44). Glucose-based peptidomimetic NK analogue.

Luteinizing Hormone-Releasing Hormone

The decapeptide amide Luteinizing Hormone-Releasing Hormone (LHRH, Table 1) [269], *p*Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2, is released from the hypothalamus and stimulates the anterior pituitary gland resulting in the secretion of the gonadotropins luteinzing hormone (LH) and follicle-stimulating hormone (FSH). LHRH, also termed gonadotropin-releasing hormone, plays an important role in the regulation of reproductive functions, thus rendering its synthetic analogues useful tools for the treatment of endocrine-based diseases like prostate and breast cancer, endometriosis, uterine leiomyoma, and precocious puperty [270]. Even though LHRH agonists proved to be useful in the treatment of the above mentioned diseases [271-273], research has also focused on the development of potent and safe antagonists.

Recently, Takeda presented a substituted 4-oxothieno[2,3-*b*]pyridine as a highly potent and orally active nonpeptide antagonist of the human LHRH receptor [274]. Again, this research program was based on the structural characteristics of a β turn suggested as the dominant conformational feature within [5-8]LHRH (Fig. (45)) [272,273].



140 141

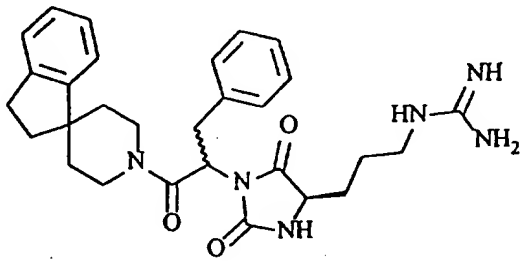
Fig. (45). β turn-derived design strategy uncovered highly active non-peptide LHRH analogues.

The β turn is considered to represent the bioactive conformation of LHRH in the receptor-bound state. Therefore, the structural element of a β turn was attempted to be transferred onto a rigid scaffold which mimics the β turn and can be decorated with the crucial functionalities, thus positioning them into the receptor-complementary orientation (Fig. (45)). For this purpose, a directed screening approach was initiated aimed to uncover compounds showing similarity to the turn template. The screening towards the inhibitory effect on the specific binding of [^{125}I]leuprolelin to human LHRH receptor [275] expressed in CHO cells resulted in the initial lead compound **140** (Fig. (45)) [274].

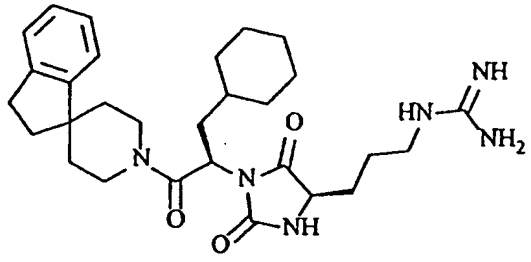
This compound was structurally compared to the hypothesized β turn arrangement and changed in order to fulfil the structural requirement imposed by that template, e.g. substituting Gly by hydrophobic D-amino acids increased activity presumably due to stabilization of the β turn by introducing a D-amino acid into the *i+1* position of the β turn. Subsequent modifications finally led to the discovery of T-98475 **141** (Fig. (45)) exhibiting an IC_{50} value of 0.2 nM for the binding to the cloned human LHRH receptor. Further, T-98475 **141** shows inhibitory effects on LHRH-stimulated LH release in functional *in vitro* and *in vivo* assays. Thus, T-98475 **141** is a good candidate of a new class of therapeutics for the treatment of LH-induced dysfunctions in sex-hormone-dependent pathologies.

C5a

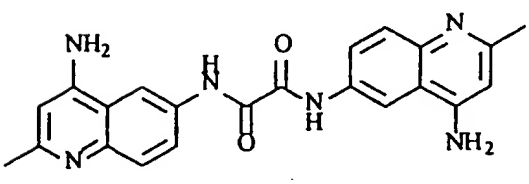
The 74 amino acid peptide C5a (Table 1) is released after activation of the complement system at sites of inflammation



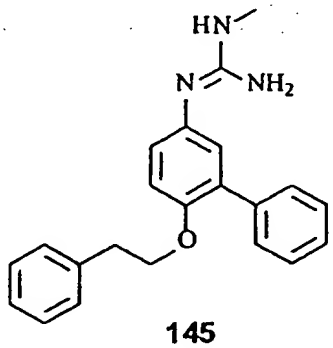
142



143



144



145

Fig. (46). C5a analogues.

by proteolytic cleavage of the complement factor C5 [276]. The hormone-like peptide anaphylatoxin, C5a, acts as chemotaxin by attracting and promoting the degranulation of granulocytes and macrophages during immune response [277,278]. Inappropriate activation of C5a results in a number of inflammatory diseases including rheumatoid arthritis [279], Alzheimer's disease [280], ischemic heart failure [281], psiorasis [282], atheriosclerosis [283], and adult respiratory distress syndrome (ARDS) [284]. In this sense, agents preventing the interaction of C5a and its receptor, C5aR, would be useful for inhibition of the pro-inflammatory function of C5a, thus, being a useful therapeutic in the treatment of chronic inflammatory disorders induced by activation of the complement system and the release of C5a [285,286]. The binding of the small protein C5a to its receptor is characterized by two interaction sites. A two-site model has been proposed localizing the major binding epitope for the ligand C5a in the extracellular *N*-terminal region of the receptor, while the second binding cavity is located in the core of the transmembrane helix bundle, obviously serving as the "activating binding site" recognizing the *C*-terminal octapeptide of the ligand [287,288]. Starting from the sequence of the native ligand a number of peptide-based antagonists were discovered which have been reviewed only recently by Wong *et al.* [289]. Obviously, the development of a nonpeptide antagonist in this filed is a major challenge since research revealed only low molecular weight compounds acting as C5a agonists or at least partial agonists over the last two decades.

Merck identified an initial lead **142** (Fig. (46)) by screening an in-house sample collection for the displacement of [^{125}I]C5a from human neutrophil membrane preparations which served for further optimization [290].

The spiroindane-bearing hydantoin **142** has been modified by introduction of a cyclohexylmethyl group instead of the benzyl residue resulting in compound **143** (Fig. (46)) which exhibits an IC_{50} value of 0.3 μM.

Surprisingly, functional receptor assays revealed that all compounds of this series with affinity for C5aR showed an agonistic potential. The only nonpeptide antagonists have been reported by Merck investigating 4,6-diaminoquinolines (**144**) [291] and Rhône-Poulenc Rorer identifying a phenylguanidin by random screening (**145**, IC_{50}=0.8 μM) (Fig. (46)) [292].

As random screening techniques have not brought the expected success, rational design would offer an alternative in the lead finding process for C5a antagonists. Based on the results of conformational studies of cyclic pentapeptide ET antagonists, BE-18257B and BQ-123 [293,294], Wong and co-workers [295,296] followed the same strategy as presented by Porcelli *et al.* [266] for the design of the SP antagonist, ITF-1565. BQ-123, *cyclo*-(D-Val^1-Leu^2-D-Trp^3-D-Asp^4-Pro^5-) and ITF-1565, *cyclo*-(D-Trp^1-Pro^2-D-Lys^3-D-Trp^4-Phe^5-) follow an identical chirality pattern of $D^1L^2D^3D^4L^5$ leading to a $\beta II/\gamma_{(i)}$ turn arrangement with L^2-D^3 in *i+1* and *i+2* position of the β turn and L^5 in the central position of an (inverse) γ turn. The strategy seems also to be applicable to C5a, since the *C*-terminal-derived C5a antagonist NMe-Phe-Lys-Pro-D-Cha-Trp-D-Arg (Cha: cyclohexylalanine) shows a well defined structure in solution in which the lysine sidechain is in close proximity to the D-arginine carboxylate. Ring closure resulted in a backbone-to-sidechain cyclized peptide, *cyclo*-Ac-Phe-(Orn-Pro-D-Cha-Trp-D-Arg-) (brackets indicate the sidechain-to-backbone mode of cyclization, Orn-NH$^{\varepsilon}$-CO-D-Arg) with an IC_{50} value of 9.28 μM for the displacement of [^{125}I]C5a from human polymorphonuclear (PMN) cells. Conformational analysis revealed a γ turn with Pro in the central position stabilized by a hydrogen bond between the flanking amino acids, Orn-CO···HN-D-Cha, together with a "pseudo" βII turn involving D-Cha-Trp-D-Arg-Orn defined by a second hydrogen bond between D-Cha-CO···H$^{\varepsilon}$N-Orn. This is consistent with ϕ_{i+1}/ψ_{i+1} and ϕ_{i+2}/ψ_{i+2} dihedrals of Trp and D-Arg (-58°/90°; 69°/-3°) confirming a β turn type II (ideal values: -60°/120°; 80°/0°) arrangement [295]. More detailed SAR studies showed that the L-Arg containing isomer is much more active than the D-Arg congener (IC_{50}=20 nM; inhibition of C5a-induced release of myeloperoxidase from PMNs). The NMR-derived solution structure reveals an inverse γ turn (γ_i) involving D-Cha-Trp-Arg stabilized by a hydrogen bond between D-Cha-CO···HN-Arg [296].

CONCLUSION

This review was intended to highlight not only the relevance of the GPCR superfamily for drug development purposes during the last decade, but also the tremendous potential of that particular target class for future medicinal chemistry programs aimed to uncover new ligands for peptide-binding GPCRs. Especially the cross-fertilizing combination of ligand-derived structure rationales with the dramatically enhanced efficiency of automated synthesis and combinatorial chemistry will enable pharmaceutical research to identify new chemical entities more rapidly. Even though we have witnessed a technology-based quantum leap forward in efficiency within medicinal chemistry in the late 1990's, the vigorous search for novel GPCR genes within e.g. the human genome has far outpaced the identification of novel endogenous and exogenous ligands. The identification of these ligands remains one of the most challenging tasks in modern pharmacology. The number of GPCRs for which endogenous or exogenous ligands are unknown today continues to increase, thus offering modern pharmaceutical research new opportunities in that entirely new drug targets associated with innovative therapeutic principles emerge. In this context, new low-molecular weight ligands for these orphan receptors will undoubtedly lead to novel insights into the complexity of numerous poorly understood human disorders. Consequently, targeted medicinal chemistry approaches towards members of the GPCR family will facilitate the understanding of the precise physiological role of orphan receptors as well as produce new compounds as qualified lead structures for clinical development.

Concluding, the field of GPCR research is clearly expected to grow dramatically due to the progress that will be made in the human genome initiative, demanding increased contributions from medicinal chemistry in order to provide new pharmacological tools as well as new leads for the development f new drugs.

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